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A NEW METHOD FOR MEASURING MACROPARTICULATE  
SYSTEMS APPLIED TO MEASURING SYNERESIS  
OF RENNETED MILK GELS

by

Jonathan R. Maynes

A dissertation submitted in partial fulfillment  
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Nutrition and Food Science

Approved:

UTAH STATE UNIVERSITY  
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1992



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Jonathan R. Maynes

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## LIST OF SYMBOLS

<u>Symbol</u>	<u>Definition</u>
$V$	velocity of fluid flow throw a porous media
$B$	permeability function of the media
$\Delta P$	pressure differential on the fluid
$\eta$	viscosity of the moving fluid
$L$	the distance the fluid must move before exiting
$V_{\infty}$	volume of expressed whey after syneresis is complete
$V_w$	measured volume of whey after a specified time
$V_0$	initial volume of milk
$V_c$	the volume of the curd
$t$	time expressed in minutes
$k$	calibration constant
$T$	the temperature of the sample
$T_0$	starting temperature of the sample
$\nabla$	gradient function
$\cdot$	dot, or inner, product vector function
$\times$	cross product vector function
$E$	electric field vector used in describing electromagnetic phenomena
$\rho_t$	charge density in the propagation media
$\epsilon_0$	permittivity constant in the propagation media
$B$	magnetic field vector used in describing electromagnetic phenomena
$\mu_0$	permeability constant of the propagation media
$J$	current density vector in the propagation media

## LIST OF SYMBOLS (continued)

<u>Symbol</u>	<u>Definition</u>
$c$	speed of light in a vacuum
$m$	the ratio of external and internal refractive indices of a particle
$n$	the refractive index of the propagation media
$X$	the size parameter of a scattering center
$\lambda$	the wavelength of the electromagnetic radiation
$a$	radius of the scattering sphere
$\pi$	pi
$\rho$	the phase lag of the radiation caused by propagating through a particle
$W_{\text{ext}}$	Energy extinguished from a light beam
$W_i$	Energy incident on a scattering center
$W_{\text{det}}$	Energy incident on the detector
$S_i$	Poynting vector, obtained taking the real part of the cross product of the electric vector and the conjugate magnetic field vector
$e_r$	propagation vector
$A$	area of integration
$C_{\text{ext}}$	extinction cross section
$G$	geometrical cross sectional area of a particle
$C_{\text{abs}}$	absorbance cross section of a particle
$I_t$	energy transmitted through a sample, equal to $W_{\text{det}}$ , used because of the familiar equation form
$I_0$	energy incident on the sample, similar to $W_i$ , used because of the Beer-Lambert usage
$Q$	extinction coefficient of a particulate suspension

## LIST OF SYMBOLS (continued)

<u>Symbol</u>	<u>Definition</u>
$a$	cross sectional area of a single particle
$A_p$	projected area of a collection of particles
$N$	the number of particles in a sample cell
$l$	the length of the sample cell
$p_s$	percentage of the fractions in suspension
$Cn_c$	the casein fraction times its index of refraction
$Sn_s$	the soluble protein fraction times its index of refraction
$Ln_l$	lactose fraction times its index of refraction
$Fn_f$	fat fraction times its index of refraction
$R$	all other solids that may contribute to the refractive index of the milk
$C_Q$	correction factor to the scattering coefficient
$\theta$	angle of integration
$i$	directional vector perpendicular to direction of propagation
$i_2$	directional vector perpendicular to propagation direction and orthogonal to $i$
$\alpha$	acceptance angle of the detector
$l_d$	distance from the particle to the detector
$T_{jik}$	statistically predicted transmission
$\mu$	transmission mean
$R_i$	variability due to replication
$S_j$	variability due to bead size
$C_k$	variability due to bead color

**ABSTRACT**

A New Method for Measuring Macroparticulate Systems  
Applied to Measuring the Syneresis  
of Renneted Milk Gels

by

Jonathan R. Maynes

Utah State University, 1992

Major Professor: Dr. Conly Hansen  
Department: Nutrition and Food Sciences

Syneresis is an integral part of cheese manufacture. The rate and extent of syneresis affect the properties of cheese. There are many factors that affect syneresis, but measured results vary because of inaccuracies in measuring techniques. To better control syneresis, an accurate mathematical description must be developed. Current mathematical models describing syneresis are limited because of inherent error in measuring techniques used to develop them. Developing an accurate model requires an accurate way to measure syneresis.

The curd becomes a particle in a whey suspension when the coagulum is cut. The most effective technique to measure particle size, without interference, is with light. Approximations to rigorous Maxwellian

theory render useable results for a variety of particle sizes. Assumptions of Fraunhofer diffraction theory relate absorption to the cross sectional area of a particle that is much larger than the wavelength of light being used. By applying diffraction theory to the curd-whey system, this researcher designed a new apparatus to permit measurement of large particle systems. The apparatus was tested, and calibrated, with polyacrylic beads. Then the syneresis of curd was measured with this apparatus.

The apparatus was designed to measure particles in suspension. Until some syneresis takes place, curd does not satisfy this condition. Theoretical assumptions require a monolayer of scattering centers. The sample container must be thin enough to preclude stacking of the particles. This presents a unique problem with curd. If the coagulum is cut in the sample cell, it adheres to the front and back surfaces and does not synerese. The curd must be coagulated and cut externally and transferred to the sample cell with a large amount of whey. This measurement technique has other limitations that may be overcome with commercially available accessories.

## CHAPTER I

### INTRODUCTION

Syneresis is a complex, poorly understood process (78). Published results are often contradictory, and much discrepancy, exists adding to the confusion about the mechanisms of syneresis. Syneresis is defined as the spontaneous separation of an initially homogeneous colloid system into two phases - a coherent gel and a liquid. There are three causes of syneresis: 1) desorption, 2) aggregation, and 3) contraction. In desorption the particles become less hydrophilic and exude moisture. Particles unite in aggregation, decreasing the volume of the gel network and forcing fluid separation. With contraction a similar fluid expulsion occurs (82). Syneresis occurs in a wide variety of foods, including dairy products (27,51). Greater control in dairy processing and machine design lies in increased knowledge of syneresis and phase separation (50).

Many factors affect syneresis, and the general trends of their effects have been studied. Inaccurate measurement techniques impair modeling syneresis. Current mathematical models are inadequate for use in controlling or predicting syneresis. All techniques used to date have inherent inaccuracies and problems. Most of these methods interact with the curd and affect the properties being measured, yielding questionable results. A better method of measuring needs to be developed.

In cheese manufacture, milk is coagulated and the coagulum cut into small pieces. Syneresis results in a curd-whey mixture. Curd can be considered a particle. Light scattering, absorption, and extinction, as well as other techniques, have been used to measure particle size. Light does not interact with particles in solution and can effectively measure curd size.

Light-scattering theory is limited to particles that are approximately the same size, or smaller than, the wavelength of light being used to measure the particle. Approximations can be used to develop theories that may be used with larger particles. Curd particles do not satisfy the requirements to be measured by scattering techniques but theoretical approximations may be invoked to enable use of absorption methods that yield accurate results. These approximations impose constraints in the design of optical devices to measure particle size.

Development of a method using light extinction techniques to measure syneresis accurately, and with less interaction than previous methods, was hypothesized. The objective of this research was to design, construct and test the system and software for a real-time, non-interfering method of measuring syneresis by using the light scattering properties of cheese curd. Specific secondary objectives that led to accomplishment of this were 1) determine necessary optical components and



relative optimal spacing of these components, 2) design and calibrate the sample vial for desired characteristics, 3) determine the optimal angle between sample and detector, 4) calibrate the equipment to establish the relationship between particle size and light transmission, 5) make preliminary measurements of syneresis and establish an error variance.



## CHAPTER II

### LITERATURE REVIEW

#### SYNERESIS OVERVIEW

##### Introduction

Syneresis is not well understood even though there is considerable information on factors that influence it (27). Syneresis occurs in a wide variety of foods, including dairy products (27). In some dairy foods syneresis is undesirable and leads to defects. For example, a quality yogurt product has a firm gel. Syneresis leads to formation of a liquid layer on the top of packaged yogurt and is a noticeable flaw (62,86). In this case syneresis must be controlled and hopefully eliminated (35,87). In contrast, syneresis is an essential requirement in cheese-making from renneted or acidified milk (63). Controlled syneresis is essential in making cheese, yet cheese may shrink after production, causing product loss.

Cheese-making is the controlled syneresis of the rennet coagulum. The quality of cheese obtained depends on the degree of syneresis (60). A general description for the production of cheese follows. Milk is heated to the desired temperature. A coagulating starter is then added that comprises about 2-3% of the volume. The vat is usually ripened for approximately 20 minutes before

addition of rennet. When the coagulum is of the desired firmness, it is cut into small pieces. The vat is stirred to keep the curd in suspension and to prevent matting. The temperature of the curd and whey mixture is gradually raised to cooking temperature. Along with the action of the rennet and increasing acidity, the higher temperature enhances curd contraction and the expulsion of moisture from the curd. Later the whey is drained from the curd and the curd is processed further (88).

Describing syneresis as a function of milk properties and process conditions is important when new methods or processing steps are introduced in cheese-making. Regulation of moisture content of the finished product implies controlling syneresis. The rate of syneresis affects processing methods and equipment usage time. The final characteristics of the cheese are dependent on the rate and extent of syneresis (86). Differences in syneresis throughout a mass of curd cause compositional differences in the cheese. Even after the cheese is formed, it may still synerese, resulting in moisture loss (117).

By controlling manufacturing and ripening conditions it is possible to produce cheese of consistent excellent quality (27). Curd firmness and the rate of whey syneresis are the most important parameters in the cheese-making process (20). The rates of curd formation stages

are important because they directly affect subsequent properties of cheese curd (68).

Some theoretical mechanisms of syneresis in dairy products are the following: 1) solubility of casein micelles changes, 2) formed network rearranges to minimize surface to volume ratio, and 3) casein micelles in the gel matrix shrink (117). In enzymic coagulation, modification of the casein and the presence of calcium are necessary for syneresis (2). Solubility must change since coagulation of the micelles is a precursor to syneresis.

### **Coagulation**

Milk coagulation is the first basic step in the cheese-making process (81). Milk coagulation is a complex process, and although much is known about it, the mechanisms of coagulation have not been elucidated completely (65). A further problem resides in the complexity of the casein micelle, the lack of detailed information on its structure, and disposition of components on its surface (78).

Milk conversion to cheese coagulum after adding rennet can be divided into three separate but overlapping stages: enzymic proteolysis, aggregation, and gelation (49,64,99). Most of the proteins in milk are found in a structure called a casein micelle. There are several theories about the structure of the micelle (96). One theory suggests that casein micelles originate by

aggregation of more or less globular particles of fairly uniform dimensions (10 to 20 nm) called submicelles (18). The casein micelle itself behaves as a gel of the many submicelles (41). Submicelles consist of  $\alpha_{s1}$ ,  $\alpha_{s2}$ ,  $\beta$ , and  $\kappa$  caseins. These are held together by hydrophobic interactions and calcium phosphate. The ratio of  $\alpha_{s1}:\alpha_{s2}:\beta:\kappa$  is approximately 3:1:3:1 but the submicelle does not have a fixed stoichiometric composition (91,96,115). The micelle exists in suspension because of the predominance of  $\kappa$ -caseins on the surface.  $\kappa$ -caseins stabilize the casein micelle in suspension through stearic interaction and provide external hydrophilic affinity (27,57). Initially micelle distribution suggests the existence of repulsion between micelles. When rennet is added to milk, the enzyme proceeds to cleave  $\kappa$ -casein. The  $\kappa$ -casein moiety is hydrolyzed by chymosin according to Michaelis-Menton kinetics (99).

$\kappa$ -casein consists of a fairly rigid globular structure comprising two thirds of the molecule to which is attached a flexible, highly solvated tail (64). The rennet cleaves the bond between Phe<sub>105</sub>-Met<sub>106</sub> (57,64). This modifies the micelle, decreasing its zeta potential and eliminating  $\kappa$ -casein's ability to stabilize the micelle (27,57). At the sites of proteolysis of  $\kappa$ -casein, the micelle surface is changed from a net negatively charged area to a positively charged area (64). When the

hydrophilic tail of k-casein is cut off, the micelles start sticking together when they collide (64,65). Calcium is involved in the intermicellar links in a complex manner (2).

Initial size distribution remains constant after addition of rennet, with changes only being observed after two thirds of the total coagulation time (49). In the simplest case, coagulation reactions can be considered bimolecular, with the aggregation rate being diffusion controlled following Von Smoluchowski principles (57,64,66,99). In reality rennet coagulation is a function of two reactions. The enzymatic reaction is a first order reaction and flocculation follows Smoluchowski kinetics (117).

Aggregation of the casein micelles will not proceed rapidly while the number of aggregate particles remains low (81). Growth of aggregates is almost entirely by addition of clusters. Growth by cluster addition leads to an open network. In only the rarest cases can clusters interpenetrate, so large holes are left in the particle matrix. As floc size increases, internal rearrangements occur to permit transfer of increasing stress (64). Gelation continues with interactions between micelle chains that cause them to become strands (66). In the final gel, micelles are only loosely packed. These loose

micelle strands entrap fat and whey serum into "microdroplets" between the strands (64).

Micelle strands in the gel network are one to four micelles (approximately 250-500 nm) thick and ten micelles long (104,117). Pore diameters are 5 to 10  $\mu\text{m}$ . Casein micelles make up about .07% of the volume (104). After the gel is formed, the free surfaces of the casein micelles are still reactive (117). Casein micelles lose their individual identity and the coagulum can be considered to be a large mesh network of casein strands (68). If the free surfaces of the casein strands touch through motion or deformation, they aggregate and cause a local stress in the matrix. The resulting pressure and contraction causes syneresis (64,105). As shown, coagulation and syneresis are not strictly separate (78).

### **Syneresis**

Rennet gels are stable if undisturbed but synerese quite strongly when cut (27). Syneresis may be regarded as a continuation of the gel assembly process (27). Syneresis is a result of a range of chemical interactions which are enabled by and dependent upon physical agitation of the curd (78). Factors that affect the rate and extent of coagulation and syneresis are not necessarily related, nor are their effects (60).

If the gel is formed completely undisturbed and if it sticks to the walls of the container in which it is



formed, it usually will not synerese. Gel stability depends on the size of the vessel and the temperature (117). Although gross syneresis may not take place, micro syneresis can. Local regions of the micelle network may agglomerate, resulting in larger pores in adjacent regions (45). But the overall stability of the curd does not change with time until the curd is disturbed (97).

Syneresis occurs as soon as the gel is cut or the surface wetted (117). The three-dimensional gel network shrinks, causing syneresis (20). Shrinkage occurs when junction points increase, enlarging the contact area, or Brownian motion brings aggregates in the gel network in contact (57,104). Either mechanism results in endogenous pressure, causing expulsion of trapped fluid (104). The casein matrix becomes more homogenous as syneresis progresses (109).

### **Factors that Affect Syneresis**

Factors affecting syneresis have been studied a great deal. Geometric factors such as size and shape of the curd affect syneresis. How syneresis is induced, the type of rennet added, and time lapse between renneting and cutting all affect the rate of syneresis. Applying external pressure, changing temperature, and the amount of stirring or agitation of the curd will alter the rate of syneresis (60). Milk composition and pH play a role in syneresis as well (117).

For many factors, conflicting effects on syneresis have been reported. The measured effect depends greatly on the method used to measure syneresis. General trends will be discussed, and where several studies are in agreement, these trends will be presented. Where only one or two studies have been done on a factor, those results are presented.

Syneresis of different types of milk is affected by various treatments differently (75). Milk produced in summer and autumn synereses faster than milk produced at other times of the year. Spring milk undergoes syneresis slowest with winter milk having an intermediate value (13). Large increases in fat concentration decrease syneresis dramatically (100).

Goat's milk exhibits much greater syneresis than cow's milk (1,117). Depending on the concentrations of  $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin, preheating milk before coagulation inhibits syneresis (60,75,78,117). Most researchers agree that addition of  $\text{CaCl}_2$  enhances syneresis but there are conflicting results (11,20,27,117). Mastitis affects the milk and yields a poor gel with decreased syneresis (60,117).

Even the genetic variants of proteins have differing effects. The extent of syneresis is greater in milks containing  $\kappa$ -casein B than it is for milks containing  $\kappa$ -casein A. Milk containing  $\kappa$ -casein B coagulates faster,



forms a firmer curd, and produces a greater yield of cheese compared to milk containing  $\kappa$ -casein A. The genetic variant of  $\beta$ -lactoglobulin has a greater effect per se than the effect on concentration (63). Milk with  $\beta$ -lactoglobulin B synereses less than milk with  $\beta$ -lactoglobulin A.

Concentrations of various milk proteins affect syneresis and coagulation differently. Coagulation time varies inversely with  $\kappa$ -casein concentration, but affects syneresis slightly (76). Reducing  $\alpha$ -casein concentration below levels found in fresh milk increases overall syneresis (76,100). Increasing the concentration does not have any effect on coagulation or syneresis. The converse is true with  $\beta$ -casein. Reducing  $\beta$ -casein concentration does not affect syneresis, but increasing concentration increases the rate and extent of syneresis significantly (76).

Ultra filtration is a method of increasing the concentration of proteins in milk that has come into wide use recently. Since desired syneresis is the controlled process of eliminating moisture from milk while retaining the solids, it follows that concentration of the milk prior to coagulation may reduce the time it takes for syneresis to proceed; hence a shorter time is needed to make cheese. Syneresis is indeed inversely related to total protein concentration. The extent of syneresis is

lessened as solids concentration increases (5,20,53,59,80,97). Syneresis ceases to occur when solids concentration reaches 35 to 40% (53). The rate of syneresis is independent of solids concentration, so total time is less (59,80). Even though the extent of syneresis is lessened, syneresis is accentuated by higher concentrations, and a larger proportion of the whey is expelled. The resulting curd has a lower moisture content, yielding drier cheese (20). Also the composition of the curd differs from that obtained from non-concentrated milk (80).

In addition to the milk composition affecting syneresis, treatments done on the milk prior to coagulation have a large impact on the results. Treatments commonly performed on milk are homogenization and pasteurization. Homogenization of milk reduces renneting time, yields a softer, more elastic coagulum, and syneresis is slower and more uniform (25,47).

Effect of the heat treatment on syneresis depends on the temperature the milk is subjected to, the storage temperature, and on how long the milk is stored prior to use. In general, as the temperature of the pretreatment increases, the rate and extent of syneresis slows and the final moisture content of the curd is higher (5,97,103). Syneresis is adversely affected by preheat treatments where the temperature is above 65°C. The more severe the

treatment, the greater the inhibition to syneresis. Levels of  $\beta$ -lactoglobulin, and to a lesser extent  $\alpha$ -lactalbumin, affect the inhibition of syneresis in the curd (75).

Immediately following a heat treatment, effects on coagulation and syneresis are erratic, but after being stored two to four days the effect of the heat treatment on coagulation and syneresis stabilizes (75). Reducing the storage temperature also reduces syneresis (67,84). By holding the milk at 2° and 6°C, the rate of syneresis decreased by 13.9 to 21.3%. At 10°C syneresis increased by 9.7 to 16.5%. These results are difficult to interpret as there is a two-fold increase in proteolytic organisms and a 3 to 3.6-fold increase in bacterial count (73). It is hard to say whether the difference was due to the increased temperature or higher number of organisms.

Syneresis is a function of pressure and resistance of the fluid to flow through the matrix (104,105). The classical treatment of flow through a porous medium is given by Darcy's equation:

$$v = (B\Delta P)/(n \cdot L) \quad (1)$$

where  $v$  is the linear flow rate,  $B$  is the permeability coefficient of the network,  $\Delta P$  is the pressure gradient within the network,  $n$  is the viscosity of the fluid, and  $L$

is the distance the fluid moves (27). The permeability of the curd increases with time, concentration, deformation, temperature, and shear strain. Permeability decreases with increasing pH and fat concentration. External pressure affects the permeability such that the larger the pressure, the greater the change in the permeability of the matrix (104). Syneresis pressure is surprisingly low (about 1 Pa), and small external pressures exert large effects (106). Hence, anything that exerts pressure on the curd will affect the exiting flow of whey.

Syneresis is initially a first order reaction with respect to flow. The net pressure depends on the whey content of the curd and volume of external whey. Holding the curd in the whey retards syneresis due to the back pressure of the surrounding whey (27,62). The greater the volume of surrounding whey, the slower the rate of syneresis (55). In actuality, removal of up to 50% of the whey has little effect on the final moisture content of the curd (7). But removal of curd from the whey causes a very rapid decrease in moisture content (106). When curd volume is reduced to approximately 70% of its initial volume, syneresis depends on factors other than the volume of whey surrounding (27).

In general the effect of coagulum rigidity on whey separation is small. At relatively low gel strength ( $<15 \text{ N/m}^2$ ), whey drainage tends to increase as rigidity

increases, but at high gel strengths ( $>25 \text{ N/m}^2$ ) the opposite is true. Syneresis does not depend on rigidity as might be expected on thermodynamic grounds (56).

Stirring the curd greatly affects syneresis and tends to minimize the effect of the previous heat treatment (55,97). When curd is stirred, syneresis is nearly complete 30 minutes after cutting (55). In addition the measured rate of syneresis depends on the amount of work done on the curd. This has proven difficult to control in small-scale experiments (62).

As with other factors, the effect of cooking temperature depends on previous treatments to the milk. For example, at  $32^\circ\text{C}$ , syneresis is slower if the curd is cut at 45 minutes after coagulation time than it is when the curd is cut at 15 minutes. At  $40^\circ\text{C}$  the same effect is not observed (97). Some studies have tried to find a linear relationship between temperature and syneresis (54,97). In general syneresis increases with an increase in cooking temperature, but the amount of increase is not agreed on (7,11,15,16,27,48,60,117). Steam injection heating of milk impairs syneresis to a greater extent than indirect heating does (89) even though there is a dilution factor to be considered.

Heating the milk in the presence of reducing sugars greatly retards syneresis. However, only  $\beta$ -casein is

affected by the Browning reaction (110). Here too the interaction of several factors is evident.

Syneresis is favored by low pH (7,27,28,97).

Lowering initial pH of the milk greatly affects the amount of syneresis and the percentage of total solids retained in the curd (16). Lower initial pH also minimizes the effects of the preheating treatment. Maximum syneresis occurs near the isoelectric point of the casein micelles, but at pH 5.5 the curd becomes very hard and rubbery (97). Developing acidity theoretically hastens syneresis by breaking down the intermicellar calcium links as well as the micellar nature of the network strands. Micelle structure breakdown gives rise to new internal forces of attraction which cause syneresis of the gel (2).

Effects caused by lowering the pH can be observed very rapidly (97). The extent of syneresis is greater as pH decreases (80). When a fermenting culture is added, lactic acid develops as syneresis proceeds, lowering the pH. This leads to solubilization of mineral constituents of the casein complex. Diffusion of lactose into, and lactic acid out of, the curd accompanies mineral solubilization (23,97). When acid development is fast, lactose is not replaced as rapidly as it is used in lactic acid formation, and more calcium phosphate is solubilized and alters curd properties (23). The pH at whey drainage



determines the calcium content of cheese which influences texture as follows (27):

Fast acid development→low pH→low  $\text{Ca}^{++}$ →crumbly texture.

Slow acid development→high pH→high  $\text{Ca}^{++}$ →rubbery texture.

Hence, the mineral composition of the curd is directly related to pH which affects texture. This example of how a factor that affects syneresis will alter curd properties typifies the complexity and interrelationship of all factors.

Syneresis requires the presence of calcium, and the effects of calcium addition in the form of  $\text{CaCl}_2$  have been studied. The level of  $\text{CaCl}_2$  added greatly influences syneresis. Addition of small amounts of  $\text{CaCl}_2$  to the milk or the curd-whey mixture increases syneresis (97,98,103). Large  $\text{CaCl}_2$  additions have the opposite effect (2,3,104). Excessive  $\text{CaCl}_2$  increases the amount of colloidal calcium phosphate; consequently, the rigidity of the gel increases (3). The extent of the effect of  $\text{CaCl}_2$  depends on other factors. Low levels of  $\text{CaCl}_2$  tend to decrease syneresis when used in conjunction with synthetic enzymes. At higher levels of  $\text{CaCl}_2$ , e.g., 2 millimolar (mM), syneresis is greater when synthetic enzymes are used in the place of rennet (28).

At higher temperatures, addition of  $\text{CaCl}_2$  increases the rate of syneresis (97). Addition of other salts, such as magnesium, sodium and aluminum chloride, also affects syneresis. Calcium is used in the curd structure preferentially over magnesium (97), but addition of magnesium and sodium have the same effect in general. Adding small quantities of salts increases syneresis, whereas higher concentrations have the opposite effect (97,98). Addition of aluminum chloride or other trivalent cation to the milk prior to coagulation retards syneresis (97,98). Syneresis increases if the salt is added after the curd is cut (98). Decrease in syneresis as salt levels increase is hypothetically due to protein-salt interactions that increase water binding capacity of the micelle (72). The greatest syneretic effects occur when salt is added to milk prior to addition of coagulant (97).

The effects of sodium citrate, sodium lactate, ethylenediaminetetracetate (EDTA), and  $\text{CO}_2$  have also been investigated (21,97,98). Small levels of sodium citrate and sodium lactate increase syneresis, probably due to the ability to bind  $\text{Ca}^{++}$ . Sodium citrate severely alters the curd structure and gel properties (97,98). At higher levels sodium citrate and EDTA decrease syneresis, but, as with salts, the extent of the effect is influenced by temperature and pH (98). Effect of EDTA addition was also



greater when added prior to coagulation (97). Adding  $\text{CO}_2$  to the milk increases syneresis and curd firmness (21).

Since addition of salts affects syneresis, what about extraction of inherent phosphates? Partial dephosphorylation increases coagulation time and reduces the extent of syneresis. The effects on rennet coagulation and syneresis appear to be attributable to dephosphorylation of casein itself and the effect on surface properties rather than gross structural changes of the micelle (76).

The unique reaction in the rennet coagulation of milk is the specific rapid hydrolysis of  $\kappa$ -casein by acid proteinase causing it to lose its ability to stabilize the micelle (27). The characteristics of curd syneresis are very similar with different enzymes (24), but the absolutes of the rate of syneresis, syneresis kinetics, and total syneresis differ (4,28). Enzymes that break down proteins more extensively result in increased syneresis rates and yield a harder cheese (86). Addition of larger amounts of rennet increases syneresis if the coagulum is cut at a consistent firmness (103). When the gel is cut after a specific time, increasing the amount of added rennet results in a firmer curd and reduced syneresis (56).

The effect of the enzyme depends on the cutting time,  $\text{CaCl}_2$  concentration, and temperature (3,97). Milk that is

held at 5°C for a time with rennet synereses faster than milk which is at the desired temperature at rennet addition (71). At 32°C syneresis is enhanced when rennet levels are increased up to 2%. Additional rennet does not affect the syneresis rate. Temperature dependence seems to decrease as temperature increases and interaction between rennet addition and temperature tends to minimize above 40°C (97). Addition of plasmin to the milk affects syneresis, depending on the handling of the milk after addition of the plasmin (77).

In summary, syneresis does not occur until the gel is disturbed (27,117). Syneresis increases as pH drops to the isoelectric point. Acidifying the milk before adding the rennet results in faster syneresis (10,15,40,60,117). The size of the pieces the curd is cut into greatly affects syneresis. The smaller the pieces the faster syneresis proceeds (11,117). Increasing external pressure enhances syneresis (117). Stirring the curd increases syneresis (10,15,27,117) and reduces the effects of other variables observed when studying syneresis under quiescent conditions (117). The coagulant (11,60), composition of the milk and the coagulation procedures that are used greatly affect syneresis (11,15). See Table 1 for a breakdown of factors and effects by different studies.

**TABLE I.** Factors and their effects on total syneresis. Numbers in the table are the reference numbers of the studies that show the effect.

<u>Effects<sup>1</sup> on Total Syneresis</u>			
<u>Factor</u>	<u>Enhances</u>	<u>Variable or No Effect</u>	<u>Inhibits</u>
Protein Conc.	27	63 <sup>2</sup> , 76 <sup>2</sup>	5, 59, 67 97, 100
Fat Conc.			27, 62, 100
Total Solids Concentration			20, 26, 27 53, 62, 80 97, 104
Enzyme Conc.	26, 62, 103	97, 104	56
pH (increasing acidity)	2, 7, 16, 23 27, 28, 62, 74 97, 104	10	26
Temperature	10, 16, 27, 54 62, 74, 97 104, 110	48	
Preheating temp.	104	7, 10	75, 84, 97 103
Storage time		91	68, 84
Storage temp.			73
Homogenization			25, 47, 67
Stirring	10, 27, 55 97, 104, 106	62, 74	
Cutting time		56	
Kept in Whey		7	10, 27, 55 62, 106
CaCl <sub>2</sub> (<.02%)	27, 62, 74 104		

<sup>1</sup> Effect of increasing concentration, amount or value of the factor.

<sup>2</sup> Effect depends on the specific protein fraction.

### Syneresis Models

The rate of syneresis decreases exponentially with time, being quite rapid at first and gradually slowing to zero (54). Many mathematical models have been developed to describe the change in the rate and extent of syneresis. All models are based on the method used to measure syneresis and incorporate the inherent problems of the measurement technique. Problems associated with measuring techniques will be discussed later.

One model, based on whey separated from the system, describes the volume of whey exuded as fitting the equation (80):

$$V_{\infty} - V_w = V_{\infty} e^{-kt} \quad (2)$$

where  $V_{\infty}$  is the total exuded whey,  $V_w$  is the volume of whey expressed after time  $t$ , and  $k$  is a constant.

Another proposed model, based on the size of the curd, described syneresis in the first 60 minutes after cutting as fitting (62):

$$\text{Log}\left(\frac{V_o}{V_c}\right) = \frac{.002t}{2.303} + 0.023 \quad (3)$$

where  $V_o$  is the original milk volume,  $V_c$  is the curd volume (milk volume - whey volume).

Yet a third model dictates that between 16 and 45°C syneresis follows (48):

$$V_w = V_o e^{-k(T-T_o) \cdot t} \quad (4)$$

where T and T<sub>o</sub> are final and initial temperatures, respectively.

It is obvious that not all of these models can be universally correct. They are contradictory and simplistic. Many factors affect the rate and extent of syneresis that are not taken into account in these models (see Table 2). Results for the rate of syneresis strongly depend on conditions and even on experimental method employed. The only conclusion can be that under constant external conditions, the rate of syneresis mostly decreases as syneresis proceeds (117). Part of the difficulty in modeling syneresis is due to faulty methods used to measure it.

#### **Methods for Measuring Syneresis**

Measurement of syneresis is difficult, especially on a small scale (78). Most small-scale studies yield results which may not relate to factory processes. Experimental conditions are often quite different from

**TABLE 2.** Factors and effects of the factors on the rate of syneresis. Numbers in the table are the reference numbers of the studies that show the effect.

Factor	<u>Effects on Rate of Syneresis<sup>1</sup></u>		
	Increases	Variable or No Effect	Decreases
Protein Conc.	20,97	59,76 <sup>2</sup> ,80	52,67
Enzyme	4,28,86	24	
Enzyme Conc.	97		
pH (increasing acidity)	97	80	
Temperature	16,54,97		
Preheating temp.			97
Preheating time			5
Storage temp.			73
Homogenization			47
Stirring	55,62		
Cutting time		56	
Kept in Whey		7	27,55 62,106

<sup>1</sup> Effect of increasing concentration, amount or value of the factor.

<sup>2</sup> Effect depends on the specific protein fraction.

those used in the factory (e.g., the curd is not stirred). In addition, measurement techniques can introduce significant errors (37,121). Poor methodology is responsible for the lack of information. Few methods are used by workers other than original authors (62). Results from different methods agree on trends but vary in specifics (117). Syneresis results obtained by various methods can not be compared as conditions were not standardized (121). Existing methods and techniques can be divided into four categories on the basis of what they measure to predict syneresis (117). The first category of measurement techniques consists of methods which measure curd shrinkage. Relatively few workers have directly measured shrinkage of the curd (97,104). Measuring the rate of change of the size (i.e., height, width, volume) or curd mass is not easy because any measuring device that touches the curd alters the pressure and environment of the curd and changes the rate of syneresis. This method can only be used to effectively study early stages of undisturbed syneresis (117).

The second category of measurement techniques includes those which measure the volume of whey exuded as syneresis progresses (97). One technique is to pour, or filter off the whey at a given time and measure the volume (1,2,25,26,28,54,59,62,74,80,94,97). The delay after cutting and before draining the curd varies from five



minutes (74) to three hours (94) after cutting. The volume of whey drained off is subtracted from the initial volume of the milk to obtain the volume of the curd. But results are not reliable as this technique has inherent problems that cannot be overcome. When the curd is separated from the whey, the endpoint is highly questionable as the whey never drains off completely (117). The curd tends to collapse when handled (78) and the environment of the curd is changed. The whey exerts back pressure in the curd. Whey removal changes the net pressure in the suspended curd. Relative lactose/lactic acid concentrations are changed, which changes the rennet activity rate.

Another technique to measure syneresis in this category is addition of a chemical agent to the whey as a tracer shortly after cutting (1,121). Tracer concentration is diluted as whey is exuded. The tracer's diminishing concentration is an indication of the syneresis that has taken place (83). When the tracer method is used, curd must be stirred to ensure uniform distribution of the tracer (8). This can have adverse effects at short cutting times, but stirring is not possible with methods that measure curd size (69).

Some tracers that have been used are formaldehyde (8,68,97), polyethylene glycol (97), Blue Dextran 2000 (69,121) and clarified whey (56,63,79). The inherent

problem involved with the tracer technique is finding a tracer that does not interfere with the process or finding one that does not adsorb onto and diffuse into the curd (69,78,97). The tracer method works best with a large amount of tracer (8). But even addition of whey to the curd-whey solution depresses syneresis, and addition of tracer has the same effect (97). The higher the level of added tracer, the more the tracer affects syneresis (3,97). As tracer adsorbs onto the curd, syneresis is predicted to be greater than it actually is (79,117). Additionally, absorbance characteristics of the tracer depend on the treatments of the milk sample and the milk's characteristics (69,97). Usually, distribution of the tracer is not uniform, leading to additional inaccuracies due to sampling (97).

One unique technique that falls into the tracer category uses the fat globules in the whey as the tracer. Ultraclarified whey is layered onto the curd and the fat globules are taken as the tracer (79). As with other tracer methods, dilution of the fat globules in the whey gives a measure of the exuded whey. This assumes that all solids that separate out of the gel phase do so as syneresis starts. Even if this is so, the solids content of the initial flux of liquid from the gel varies with the rigidity of the gel (56). Solids may continue to go into solution as syneresis progresses (117). Tracer dilution

does not obtain absolute measurements of the rate of syneresis (79).

The third category involves techniques which measure the dry matter of the curd. The curd is removed from the whey, blotted dry, then weighed (16,117). This method has the same problems as the tracer method and the direct size measurement methods (117). It is difficult to separate the whey from the curd. As the curd is dried, it immediately exudes more whey, thereby making the measurement inaccurate. For example a .1 mm layer of whey on a 5 mm diameter curd leads to a relative error of 15% (117).

Category four consists of measuring the changing density of the curd. Whey loss is calculated using the changing specific density of the curd. As with the directly measuring size, measuring the specific density has the same inherent problems (97,117).

There have been other attempts to measure syneresis, but with less reliable results than for the methods previously discussed. One of these methods was estimation of syneresis by the decrease in curd firmness as whey collected around a suspended pendulum (70). Another researcher tried to use NMR characteristics of the changing gel. But at the inception of syneresis the signal became confusing, with uninterpretable results (57).

## Conclusion

Syneresis is a complex mechanism. With better methods for measuring, we will be able to develop a usable model for syneresis. This will enable us to predict the effects of different factors in cheese-making.

## Particle Sizing and Light Scattering Theory

### Introduction

For many years people have been trying to measure a multitude of particles. Particle sizes traditionally measured range from atomic up to 1000  $\mu\text{m}$  (19). Over 400 methods for determining the size of particles have been developed (90). Techniques for measuring include optical microscopy, electron microscopy, sieving and sedimentation, elutriation, permeability, adsorption, measuring diffraction rings, turbidimetry, color, total scattering, scattering at angles, forward scattering, and photoextinction (19). Many of these techniques involve separating the particle from the solution or putting pressure on the particle. The optical microscope remains the basic instrument for particle sizing (120). See Table 3 for a further breakdown of limitations on the above mentioned techniques.

Light interaction techniques are especially suited for non-intrusive application (6). Techniques that use light can be grouped into three broad categories: non-

**TABLE 3.** Particle size measurement techniques, limitations, and applicable size ranges. None are adequate for measuring curd.

Technique	Limitations	Size ( $\mu\text{m}$ )
Optical microscopy	Tedious	.2 - 100
Electron Microscopy	Expensive	.01 - 5
Sieving	Variable	50 - <1000
Sedimentation and elutriation	Depends on Technique	2 - 50
Permeability		.5 - 100
Adsorption		.01 - 50
Light scattering		.05 - 1
Turbidimetry		.05 - 50
X-ray diffraction		.01 - 10

optical systems that measure in the range .05 to 1000  $\mu\text{m}$  (e.g., integrating spheres and scattering instruments), non-imaging optical systems that measure .1 to 1200  $\mu\text{m}$ , and imaging techniques used in the range 1 to 1500  $\mu\text{m}$ . This latter technique is especially useful with non-spherical particles (95). Imaging systems use cameras, television, computers or holographic techniques to obtain parameters used to evaluate particle size (102). Imaging techniques are restricted to analyzing particles one-tenth the size of curd.

### Light Interaction Theory

Since light interaction is a non-intrusive way to measure particles, it deserves consideration for measuring curd particle size. There are many theories and approximations applying to various particle sizes. To use light scattering for studying syneresis we must first review the theory of how electromagnetic radiation interacts with particulate matter and the constraints in our present application.

All problems in theoretical optics are problems in Maxwell's theory and should be treated as such when a full, formal solution is required (107). Maxwell was the first to describe mathematically the interaction of electric and magnetic fields completely. His four equations, written below, are the basis of any study of the subject.

$$\nabla \cdot E = \frac{\rho_t}{\epsilon_o} \quad (5)$$

$$\nabla \cdot B = 0 \quad (6)$$

$$\nabla \times E = -\frac{\partial B}{\partial t} \quad (7)$$

$$\nabla \times B = \frac{1}{c^2} \frac{\partial E}{\partial t} + \mu_o J \quad (8)$$

where **E** and **B** are the electric and magnetic field vectors, respectively,  $\rho_o$  is the charge density, **J** is the current



density vector,  $\mu_0$  is the permeability constant, and  $\epsilon$  is the permittivity constant (61). These equations provide the relationships of the electric and magnetic fields both inside and outside a boundary (52). This boundary may define an infinite plane, an "infinite" cylinder such as a wire, a sphere, or any number of regular and irregular shapes. Maxwell's equations include characteristics of the interfacing media. The indices of refraction, the comparative conductances, the tendencies to induced magnetic and electric moments, etc. are all accounted for in Maxwell's equations. All electric and magnetic phenomena can be described by these equations with the appropriate approximations and constraints.

Two classes of theory are useful for sizing of particles in a liquid medium such as curd in whey: light scattering and light extinction (85). In solids and liquids, the interactions of light with charged particles are not independent and become extremely complex. Rays hitting or passing along a particle give rise to two distinct phenomena, both of which are considered scattering: 1) reflection and refraction and 2) diffraction (107). Scattering occurs when light encounters an abrupt change in the characteristics of the medium (46). Charged particles in molecules that form the material oscillate, forming dipoles (101). The polarizability of a macromolecule is enormous when



compared to the polarizability of a solvent molecule, and macromolecules move much more slowly than solution molecules. Macromolecules are far more efficient light scatterers than individual solvent molecules (9). Boundary conditions limit application of scattering to particles from .1 to 4.0  $\mu\text{m}$  in diameter. Therefore, several people have developed theories that yield good results under different constraints. Important parameters in all light scattering problems are 1) the relative refractive index  $m = n_1/n_2$ , 2) the size parameter  $X = 2\pi r/\lambda$  where  $a$  is the radius of the sphere, and 3) the phase lag  $\rho = 2X \cdot |m-1|$  due to light propagation through the particle (111,113). Some theoretical assumptions extend the theory for particles up to 50  $\mu\text{m}$  (36) (see Table 4). This is still less than one-hundredth the average curd size.

**TABLE 4.** Several light scattering theories and approximations. The approximations permit evaluation of larger and more complex situations with more intuitive results.

<u>Theory</u>	<u>Approximations</u>
Mie	None
Rayleigh	$X \sim .2, m \ll 1$
Schoenberg-Jung	$X \sim .6, 1 < m < 1.3$
Rayleigh-Debye	$0 < X < 1.2, 1.05 < m < 1.2$
Jobst	$\rho < .4, X > 20,  m-1  < .01$
Heller	$\rho \sim 2, X \rightarrow 8$
Van de Hulst	$X > 2000, \rho > 300, Q=2$

A closer look at some predominant theories is necessary to understand the effects that curd may have on light. Rayleigh developed a theory to describe scattering of light through the atmosphere. The assumptions of the Rayleigh theory are 1) the light interacts with each particle only once, 2) the index of refraction of the particle is close to 1, 3) the phase shift of the light through the particle is small, i.e., the particle is small compared to the wavelength, usually considered as  $r \ll \lambda/2\pi$  (19,107).

Another theory, extended to a collection of identical particles, is commonly called the Mie Theory. Gustav Mie developed this theory to deal with a colloidal suspension of gold particles in water (14). Looking at the energy aspects of the system, the detected energy is equal to the energy put in, less the energy extinguished. Or:

$$W_{\text{det}} = W_i - W_{\text{ext}} \quad (9)$$

where

$$W_i = -\int S_i \cdot e_r dA \quad (10)$$

and

$$S = \text{Re}(\mathbf{E} \times \mathbf{B}^*) \quad (11)$$

$W_{\text{det}}$  and  $W_{\text{ext}}$  are defined similarly to  $W_i$ , the difference is the  $\mathbf{S}$  vector considered.  $W_{\text{ext}}$ , the extinction of the incident beam, will be the same in the forward direction after being scattered by many randomly oriented, irregularly shaped particles as it would be for a collection of spherical particles of the same projected area (44). The ratio of the incident to extinguished energy results in units of area. Use this ratio to define an extinction cross section:

$$C_{\text{ext}} = \frac{W_{\text{ext}}}{W_i} \quad (12)$$

It can be shown that when viewed at infinity this value goes to:

$$C_{\text{ext}} = 2G \quad (13)$$

Where  $G$  is the geometric cross sectional area of the particle (14).

Mie theory gives the rigorous pattern of scattering and diffraction combined, but the mathematics become involved and difficult to interpret. It yields the exact scattering solution of a plane wave by a homogenous sphere restricted to a collection of spheres that are about two or three wavelengths in diameter. Approximations of Mie theory also hold for opaque particles of larger size (39). Most methods that depend on light scattering or

transmission are only applicable to a narrow size distribution. They yield values for average size, not a size distribution (19). When particles are large compared to the wavelength, intraparticulate interference must be included in calculations of scattered intensities and spectra (9). Using light scattering alone the solution for a sphere of arbitrary size and character defies analytical description (101). Normally when particles are smaller, less light is absorbed, and increasing particle surface increases the amount of scattered light (46). Frequently, a short route towards physical insight must be preferred to deduction from a given set of equations (107).

Because of complexity larger scattering bodies are characterized by various macroscopic quantities. For large particles the rigorous scattering theory has disadvantages. Often the results are very sensitive to small changes in size or refractive index of the sphere (111). Reflectivity, transmissivity, and index of refraction are material constants that are actually the statistical averaging of millions of coupled microscopic scatter events (101,108).

For particles much larger than the wavelength of light, the simplified theory of Fraunhofer diffraction, reflection, and refraction may be used (107). Fraunhofer theory may be invoked when the light source and the

detector are far from the scattering body. Under this condition reflection, refraction, and diffraction are separable only if the particle is very large (52). Reflections may be specular or diffuse. Only a surface area that greatly exceeds the wavelength can exhibit diffuse reflectance (107). The entire pattern in the Fraunhofer limit consists of two parts, a very intense central lobe due to diffraction and the less intense radiation in all directions due to optical properties of the particle (107). The major diffraction nodes are scattered in the forward direction at angles so small that it is almost impossible to separate them from the transmitted beam. The intensity of the Fraunhofer diffraction depends on the form and size of the particle, but it is independent of its composition or nature of the surface (107).

If we assume that we cannot separate the scattered light from the beam, our equation becomes:

$$C_{ext} = C_{abs} \quad (14)$$

In this application: Extinction = scattering + absorption (107). It has been shown that suspensions that appear predominantly white actually absorb very little of the incident light (46). Defining  $C_{abs}$  as the apparent absorbance of the curd, the amount of light removed from

the incident light beam is proportional to the presented area of the curd.

It has also been shown that the forward diffraction for an assembly of non-spherical particles in random orientation would be equal to that for an assembly of spheres of equal projected area (107).

In the light extinction technique the light intensity is measured at or near  $0^\circ$  (112). Total extinction by a particle that is considerably larger than the wavelength may be found in two ways. Analyzing the energy aspect, all energy falling on a particle is scattered or absorbed. The particle removes an effective area  $G$ . The diffraction of the light at infinity removes an additional effective area  $G$ . This give  $C_{\text{ext}} = 2G$ . The assumptions are 1) all scattered light is removed from the beam, and 2) observations are made at a great distance, i.e., far beyond where a shadow can be distinguished (107).

The energy that does not emerge after refraction is lost by absorption inside the particle (107). The optical density of a solution is proportional to the diameter of the particle times the extinction coefficient. Written out the equation looks like (112):

$$C_{\text{abs}} \propto d^2 Q \quad (15)$$

The ratio of intensities of incident light versus transmitted light is related to particle projected area by the Beer-Lambert equation (19,30,85):

$$\ln \frac{I_t}{I_o} = -QA_p Nl \quad (16)$$

where  $Q$  is the extinction coefficient,  $A_p$ =projected area of each particle,  $N$  is the number of particles, and  $l$  is the path length. At infinity  $Q \rightarrow 2$ , and in the near field  $Q \rightarrow 1$  (19).

The Beer-Lambert law of absorption uses the same assumptions as Fraunhofer diffraction, namely that light cut off is proportional to the projected area of the particles, irrespective of size or composition of the particle (19). For a particle with a size of 20 or more times the wavelength, it is possible to distinguish fairly sharply between the ray incident on the particle and the rays passing along the particle (107).

Beer-Lambert theory has been used successfully in applications from spectroscopy to turbidimetry. Turbidimetry is based on the apparent absorbance, defined as the extinction, rather than the actual absorbance as spectroscopy is. The optical requirements for turbidimetric determinations from extinction measurements are more strict than for normal absorption measurements (30).



If the measurements of the optical density of a turbid system can be performed under ideal conditions, the total light scattering coefficient  $Q$  is the only theoretical value needed to interpret the measurements (113). The most frequent difficulty is the failure of Beer's Law. The optical density increases to a smaller extent than is proportional to the concentration of the particles. This is usually due to multiple and dependent scattering (112). In turbidimetric analysis, multiple scattering is nearly always present. The particles receive light scattered by other particles besides radiation from the incident wave (113). Interference between waves scattered by different particles takes place when light is scattered by a number of particles (9). The overall interference effect is zero when particle distribution is random (113).

### **Optical Properties of Milk**

Light interactions with curd, and predicting the way light is scattered, absorbed, or transmitted, defy analysis. Curd can be viewed in either of two perspectives. On a microscopic scale it can be considered as a collection of scattering centers, namely the casein micelles. A second approach treats the entire curd particle as a single entity.

The white appearance of milk is primarily due to scattering of the incident light (107). Absorption of

visible light by milk is weak. The riboflavin in milk serum absorbs a small amount of light, lending a yellow-green color to the whey. Milk fat contains carotenoids that also absorb in this wavelength region, contributing a slight yellow color to milk (116). An average refractive index for milkfat is 1.4628. The index of refraction is a function of temperature and wavelength (114). For example, at 40°C, and  $\lambda = 589 \text{ nm}$ , the refractive index of fat decreases to 1.4585 (33). Casein micelles affect the refractive index of milk, but scatter less than the milkfat, probably due to the inexact nature of the surface of the micelle (116).

The refractive index of the total milk is a function of the refractive indices of the individual components. A change in the refractive index of the milk can be described by:

$$100\Delta n = p_s(Cn_c + Sn_s + Ln_l + Fn_f + R) \quad (17)$$

where  $Cn_c$  is the casein fraction multiplied by its refractive index,  $Sn_s$  is the soluble proteins with their index of refraction,  $Ln_l$  is the lactose fraction etc.,  $Fn_f$  is the fat etc., and  $R$  is the residue. The increase in refractive index of a solution over that of a pure solvent is directly proportional to the solute concentration expressed on a weight per volume basis (31). A relative

change in the proportion of fractions results in changes in the refractive index of the milk.

The casein micelle is roughly spherical (91,96) and largely determines the behavior of milk during processing (91). Since it is essentially a particle, many researchers have tried to characterize its size with several techniques including light scattering. Light scattering by small spherical particles is well described and has been previously discussed. Light scattering by micelles can be predicted if the micelles are assumed to be spherical particles. Determination of micelle size is uncertain with light scattering because casein micelles are not smooth, solid spheres, and they exist with a size distribution (93). The average radius of the micelle is variable. It may vary two-fold from the same cow at different milking times and different lactation times (42). It varies with season, the largest micelles occurring in the winter months (56). It also varies from cow to cow (41).

Casein micelles are very porous, composed of more water than proteins (18,115,116). Sometimes micelles themselves may aggregate, producing irregularly shaped bodies, which consolidate and form giant micelles which behave as a single entity (17,41). The results of light scattering, and other techniques, indicate that the casein micelle may be adequately described as a homogenous sphere

(45), and light scattering is a useful tool in describing and measuring casein micelle size.

Using light scattering, clipped autocorrelation of scattered laser light, wave length dependent turbidity and electron microscopy, 95% of the micelle mass occurs in the particles that fall in the range of 40 nm to 220 nm, with a few as large as several  $\mu\text{m}$ . The mean size is between 80 and 120 nm (12,17,43,44,58,61,91,92,93,96,116, 118). The individual micelle approach satisfies requirements for consideration under Mie theory as micelles vary from 150 to 600  $\mu\text{m}$ . This approach has many hazards and pitfalls. Primarily, the concentration of the casein micelles is such that multiple scattering must be considered. In coagulating milk the micelles begin to adhere to each other, forming chains and eventually polymerizing. Immediately the conditions of scattering change and position dependency becomes a factor. Analyzing scattering from individual micelles to measure syneresis would be futile. Restrictions are overwhelmingly surpassed. This leaves large particle assumptions of the Fraunhofer theory as the only possibility.

### **Changes with Coagulation**

Once rennet acts on the micelle, cleaving the macropeptide from the  $\kappa$ -casein, and agglomeration begins, the scattering characteristics change dramatically. At up to 60% coagulation time, micelles are widely dispersed in

the milk (34). At approximately 60% coagulation the viscosity of the milk begins an exponential rise, and the enzymic reaction is 86% complete. At 80% coagulation time micelles tend to group with some aggregation (34). Small aggregates flocculate and are often irregular and elongated. The agglomerated particles rapidly exceed the wavelength and scattering theory changes drastically. The aggregates form a network with pores several microns in width. Fat globules are trapped and usually do not become an integral part of the micelle network (116). At 100% coagulation time most micelles are linked together into chains with random orientation (66). At 130% coagulation most micelles are aggregated with extensive networks appearing. By 200% coagulation time a loose network is evident and at 300% coagulation the network consists of strands five micelles thick with interstitial spaces 10 micelles across (34). The change in aggregate size distribution with time and eventual appearance of a network suggests that the gel was assembled by linkage of one micelle at a time to preformed chains (34).

When the network begins to form the gel, light scattering takes the form of being scattered from infinitely long, thin rods with random orientation. The exact mathematical solution becomes unsolvable.

The light illuminating a curd can pass by or through without interaction, diffracted around the edges,

absorbed, reflected or refracted by the components of the gel network. The diffraction caused by very large particles results in very small angles and is inseparable from the transmitted light. We define the apparent absorbance of the curd as that light that interacts with the network in some way and does not reach the detector. The light that is detected is that light which did not interact with the curd, i.e., it is transmitted. The major question then becomes, "how much of the light is transmitted through the curd?" If the amount is negligible, then the transmitted light is in direct proportion to the cross sectional area of the particle (see Figure 1). This is the same assumption that underlies turbidity analysis. Interestingly, the turbidity of undiluted skim during coagulation obeys Rayleigh theory. The relative increase in the scattering intensity is proportional to the relative increase in average particle weight (22).

One of the basic premises underlying electromagnetic-particle interaction theory is that the illuminating light field is much larger than the particle size. Turbidimetry uses these same approximations but has usually been limited to very small particles. The reason for this is that it has been impossible to achieve a relatively large, collimated light source until the advent of the laser.



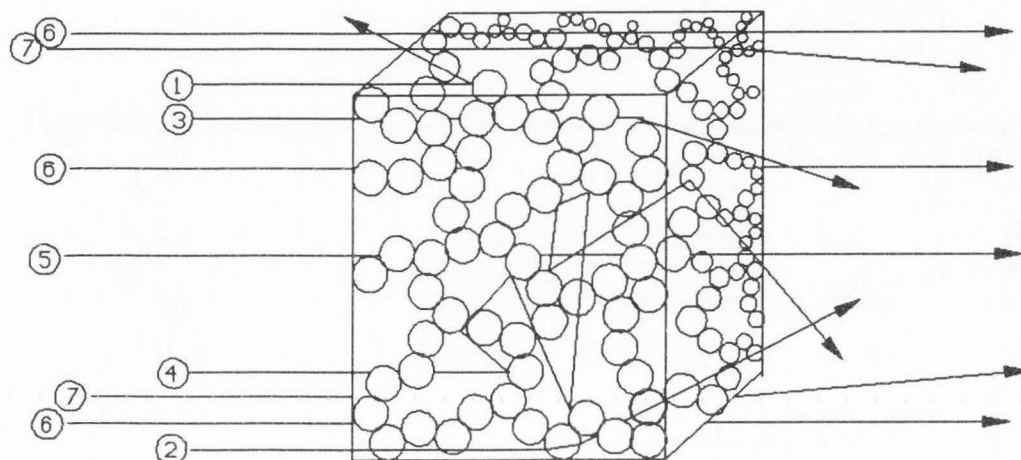


Figure 1. Light interaction with curd may occur by reflection (1), refraction (2), diffraction (3) or several interactions (4) with the micelle chains. Light may also pass by (5) or through (6) without interaction or diffract around the curd (7).

Through the use of lasers, intense, collimated, light beams that are large relative to large particle sizes can be achieved. But lasers are usually monochromatic and turbidimetric methods employing a single wavelength provide an exact particle diameter only when applied to an emulsion in which the particles are all the same size (32).

In describing the interaction of particles with a light beam Beer and Lambert developed separate, complementary theories. Since that time the theories have become known as the Beer-Lambert law. They describe effects of concentration of a particulate system (Beer's Law) and the effect of path length (Lambert's Law) on the intensity of the transmitted light beam.



The basic premise underlying this method is that the amount of light transmitted is directly dependent on the total area of the particles in the sample cell that is presented to the beam. Beer-Lambert theory is based on this premise but is traditionally used for molecular systems. In this application the equation takes the form  $I = I_0 e^{-kna}$  where  $n$  is the total number of particles, and  $a$  is the area of a single particle.

The ratio of  $I/I_0$ , or transmission, depends on the total presented area of the suspended particles. If the sample cell length is less than multiple particle diameters, then  $l$  becomes one and the equation becomes:  $I = I_0 e^{-kna}$ . This is the form of the equation that was tested.

### CHAPTER III

#### EQUIPMENT DESIGN OBJECTIVES

##### General Considerations

The major considerations in the design of this apparatus were 1) the measurement technique should not interfere with the sample in any way, 2) the conditions of the sample should approximate manufacturing conditions as closely as possible, and 3) the technique should yield accurate results.

As previously discussed, light has been used extensively in conducting measurements where non-interaction is essential. Some of the design criteria for a device to measure macroparticle size are unique in measuring curd size. The illuminating light must be much larger than particle size. Because of the particle size, the illuminating light beam must be very large, with sufficient intensity to be detected after passing through the sample. The source must be intense enough to be spread out to meet this criterion. The light source used for the experiment must have adequate power and must be collimated. The curd must be kept in suspension, yet the agitating mechanism cannot interfere with the light beam. The detector must be shielded from stray scattered light.

### Coagulation

As the micelles link and the network firms, the optical properties of the curd change. It has been shown that the percent of apparent absorbance of a milk solution that has rennet added to it initially decreases, then increases. The curve asymptotically approaches a maximum after about 30 minutes. The actual curve and rate of asymptotic approach is dictated by the amount of rennet added, the substrate characteristics, and the treatments to which the substrate has been subjected (65). Since there is obviously some percent of the incident light that is transmitted through the sample, this amount may change with amount of coagulation. Therefore, it was necessary to design a method to determine the effect of this change on the results.

### Sample Cell Design

The apparatus was designed so that sample conditions matched cheese manufacturing practices closely to obtain applicable results. The overriding concerns were that the curd remain in suspension in the whey and that the curd would be agitated. Suspension of the curd was also required to obtain uniform illumination of the curd by the light beam.

### Apparatus Design

The main feature of turbidimetry as compared with absorption photometry is that the primary light beam is attenuated by scattering. Preventing the scattered radiation from reaching the detector is desirable. Moreover, secondary scattered light should be kept out of the sample volume (112). The construction of turbidimetric instruments with a very small acceptance angle often requires the use of large spectral bandwidths to maintain beam intensity (113).

A laser could provide the needed intensity with proper collimation to be used in large particle measurements. In spectroscopes various light sources are used, from mercury discharge lamps to tungsten filament bulbs. The light from the lamp passes through a series of filter slits to attain the necessary collimation. From a common 40 watt bulb, if we permit a divergence of 1 radian, we get a net beam power of .6 mW. Inexpensive lasers are available that attain beam powers of 5 mW with a beam divergence of 1.0 mrad. An inherent problem with using lasers as a radiation source is the non-uniform intensity profile across the beam (38). This means the beam must have a diameter of approximately 10 cm. After the light beam passes through the sample, the light must be collected and measured. Stray and scattered light must be filtered out to enable determination of particle effects.

Using laser diffraction particle sizing, if either particle number density or optical path length become too large, multiple scattering becomes important and Fraunhofer theory no longer applies (38). The correction  $C_Q$  for scattered radiation reaching the detector, to be subtracted from  $Q$ , is:

$$C_Q = X^{-2} \int_0^{\alpha} [i_1(\theta) + i_2(\theta)] \sin\theta d\theta \quad (18)$$

and calculation is tedious. It is possible to make  $C_Q$  negligible by making the acceptance angle enough. For  $m \rightarrow 1$ , the condition is  $X\alpha < .2$ . If  $m$  is greater than 1, the requirement  $\alpha < .07$  rad is sufficient (113).

Fraunhofer diffraction spreading is small if  $\lambda l < d^2$  where  $l$  is the distance from the particle to the detector, and  $d$  is the diameter of the particle (119). Measured turbidity of a sample can vary unless precautions are taken to ensure a small subtense angle by the detector (29). The acceptance angle  $\alpha$  is defined as half of the sum of the divergence of the primary beam and the angle subtended by the detector with reference to the center (112). For turbidimetry measurements  $\alpha$  should be less than  $3^\circ$  (112).

To reduce stray light, experiments need to be conducted in a light-restricted environment. Hence, the beam expander, experiment volume, the light collector, and detector should all be enclosed to prevent incidental

light. Yet there must be easy access to the sample location. This can be accomplished by having an experimental housing with a light curtain to which the collector and expander are bolted.

## CHAPTER IV

### MATERIALS AND METHODS

#### Sample Cell Design and Construction

The curd had to remain in suspension in the whey and the curd had to be agitated. Suspension of the curd is also required to obtain uniform illumination of the curd by the light beam. These requirements dictate the shape of the sample container and the subsequent method of agitation. Commercially the milk is poured into a large rectangular vat and the curd-whey mixture is stirred. But stirring the curd would interfere with any attempts to make measurements using light, as the stirring instrument is likely to be detected and cause fluctuations as it passes through the light beam. The only thing that could be in the sample cell is the sample itself. The sample had to remain in the light path while it was agitated. Agitation was accomplished by rotating the sample cell in the light beam. The sample cell had to have transparent ends to permit transmission of the light.

Several sample cells were tried and a best design chosen. The initial sample container was a glass tube 8 inches long with an internal diameter of 2.9 inches and an external diameter of 3.2 inches. The material was decided upon for the sake of allowing measurements of the light at angles to the propagation direction. Threaded plastic



rings were cemented onto the glass, which allowed a glass endplate to be held in place by mating rings. These external rings were also the contact points between the sample cell and the rollers.

The second sample cell was made from machined aluminum with aluminum rings that bolted over the glass to keep the cover plates in place and prevent leakage. The extreme external diameter was four inches, with an internal diameter of three inches, and a sample volume length of one inch. This cell was designed with a sample port to permit extraction of whey sample during experimentation.

The final cell was machined aluminum also. It had a three inch internal diameter and external diameter of 4.5 inches, and a sample thickness of 1 cm. In each face was a groove for the glass plate to rest in. The glass was held in by an aluminum ring that bolted to the cell body (see Figure 2). The cell body rested directly on rollers.

### **Equipment Design and Construction**

The housing was a sheet metal box with an opening in the front. The front had five rubber panels that keep stray light out of the experiment yet permit easy access for putting the sample in and taking it out of the light path. Since the light beam had to be much larger than

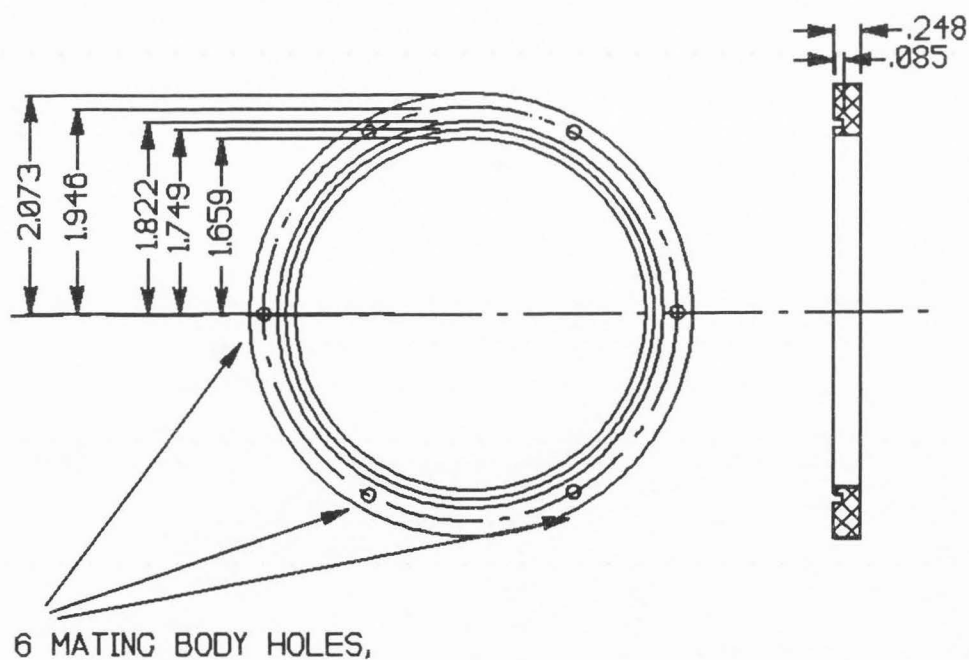
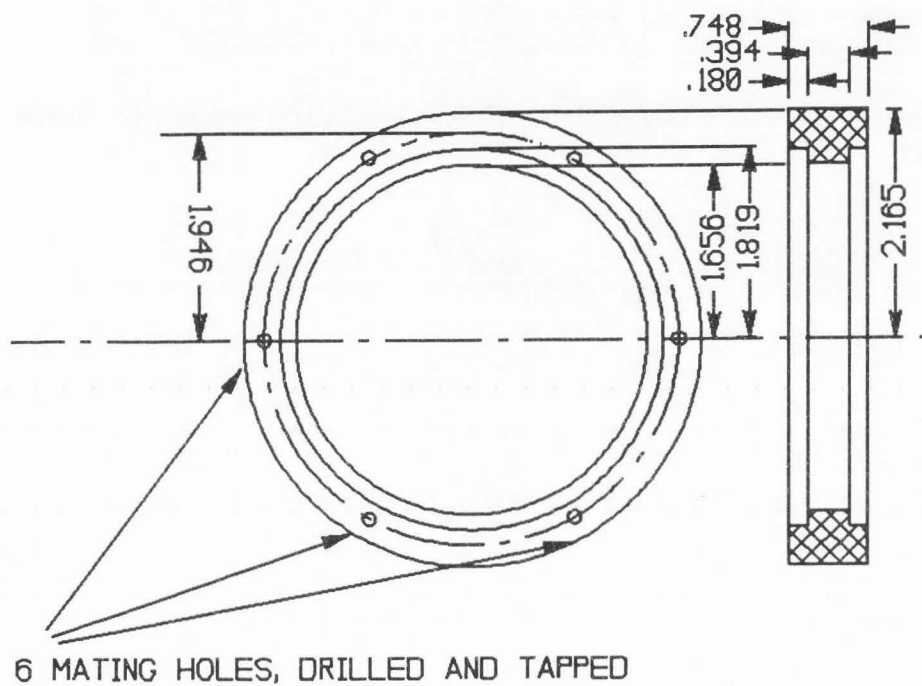


Figure 2. Sample container design. Two mating rings bolt onto the faces of the cell body to hold glass cover plates in place. All measurements are in inches.

particle size, it was arbitrarily decided that a beam diameter 10x greater than the curd diameter was adequate. A 5 mW helium-neon laser manufactured by Uniphase (Manteca, CA) was purchased. The operating wavelength was 632.8 nm. Net power output fluctuated by 5% after a 12-hour warmup. This necessitated making two measurements for each sample: one to obtain the current power output of the laser, the second to determine the sample characteristics.

The light beam was 1.1 mm as it exited the laser. The beam needed to be expanded approximately 100x. The beam expander was constructed with lenses from Edmund Scientific (Barrington, NJ) and PVC tubing. The beam expansion was a two-step process involving two pairs of lenses that expanded the beam, then recollimated it. Lenses with one planar surface were used to minimize distortion and ionization possibilities. The lenses were (in order) a 15mm x 15mm plano-concave (PCV), and a 25mm x 175mm plano-convex (PCX), a 17mm x 30mm PCV, and a 98mm x 250mm PCX. All measurements are of the lens diameter followed by the focal length of the lens.

Seats for the lenses were machined into the plastic tubing to ensure the lens would be perpendicular to the beam axis, thus minimizing off-axis effects. The distance between the lenses of a pair was adjusted so they would have a common focal point. An expansion of 90x was

effected on the laser beam to achieve a final beam diameter of 10 cm. The expander was then bolted to the experiment housing and mounted in a radial adjustment.

Often a light beam is distorted as it passes through a series of lenses. Therefore, the intensity profile of the light beam needed to be measured to ensure that the illumination field was fairly uniform. Measuring the profile was done rather simply. Poster board was cut to fit the bolt arrangement in the interior of the apparatus wall immediately prior to the detector. This was marked for the center of the beam and a circle with the same diameter as the sample cell was cut out of it. A square was drawn around the circle as a reference frame. Ten pieces of black construction paper were cut to fit this frame. A hole 0.25 inches in diameter was punched in each sheet. The location was based on a predetermined grid point. The intensity of the light was measured at thirty-seven points. The grid spacing was .5 inches. The thirty-seven points used to analyze the beam were at intersections of grid lines in the pattern depicted in Figure 3.

The beam collector consisted of a 98mm x 250mm PCX lens, a 600um pinhole spatial filter, and a 15mm x 22.5mm PCX lens. The pinhole spatial filter was located at the focal point of both lenses to filter extraneous light. The beam was then focused onto the detector. The detector

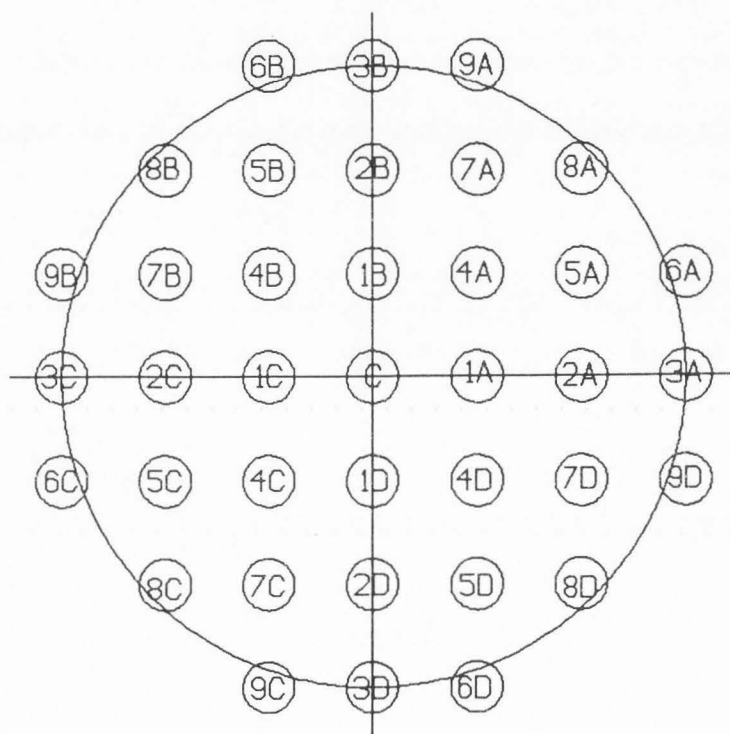


Figure 3. Beam profile sample plot. Each circle represents an observation point. The outer circle represents the extent of the sample cell.

was an Ealing Electro-Optics photodetector. It was coupled to an Ealing Electro-Optics Research Radiometer (Holliston, MA) by direct cable. The detector head had a diameter of 1.5 inches by 1 inch thick. The radiometer had an RS-232 port which was used to interface directly with the computer. Readings were made every one-half second.

The computer used was a Leading Edge, model DC-2010, IBM compatible computer. The measurements were placed directly into a file for later analysis.

The drive system consisted of a motor, reduction gears, pulleys, and roller shafts. The motor was a Talboy's Engineering Corp. 1/18th horsepower variable speed electric motor (Emerson, NJ). The motor shaft turned up to 7500 rpm and was controlled by a rheostat. The motor turned a shaft coupled to a series of reduction gears for a net reduction of 300:8. This gave a sample cell rotation of up to 90 rpm. The motor was mounted below the table and a vertical shaft was used to bring power up to the rollers that the sample rests on. Figures 13 through 21, in appendix A, are drawings of the apparatus parts and assembly.

#### **General Experimental Procedures**

The laser and detector were started up at least one half hour prior to taking data. This allowed them to warm up and equilibrate prior to running samples. Prior to taking data the drive mechanism was started and allowed to run several minutes to warm up. A blank of distilled water in the cell was run first to determine the absorption due to the glass of the cell and the water of the sample. After filling, the cell was placed on the rollers and the data collecting initiated. Data was collected for 1 minute to ensure a good data sample.

The sample container was prepared by placing a glass plate in one side of the cell and fastening a retaining



ring over it. Then the cell was inverted and filled with water or the sample. Finally a cover glass was put on the top face, taking care not to entrap air into the cell, and bolted in place. This process was repeated for every type of bead or curd/whey sample.

The transmittance of the sample was calculated by dividing the sample measurement by the laser output measurement. This value was divided by the transmission of the blank to factor out the apparent absorption of the cell and isolate the apparent absorption of the sample.

### **Bead Experiments**

Each sample was prepared by filling the cell with distilled water and counting the appropriate number of beads into the cell. The transmittance of the sample was measured, immediately followed by a measurement of the laser output. Every experiment was set-up as a type of randomized block experiment. After a block of samples was run, the transmittance of a blank was again measured.

### **Transmittance Versus Total Cross Sectional Area**

Since the assumption was based on the total area of the particulate suspension, various numbers of polyacrylic beads were used to obtain various total cross sectional areas. To test if the results were independent of bead size, 8 mm and 6 mm beads were used. A split-plot,



randomized block experiment with two factors, bead diameter as the whole plot and total area as the subplot, was used. The experimental design is outlined in Table 5, and the entire experiment was replicated twice.

The number of beads used depended on the cross sectional area of each bead. The beads used were octagonal with a three-fold axis of symmetry. The area presented to the light beam by each bead was calculated as depicted in Figure 4.

**TABLE 5.** Constant total cross sectional area experiment design. Transmittance is tested against total cross sectional area and bead diameter with different numbers of beads of different sizes constituting the total area.

Total Cross Section Area (mm <sup>2</sup> )	<u>Number of Beads</u>	
	6 mm	8 mm
5.3	18	10
10.6	36	20
15.9	53	30
21.2	71	40
26.5	89	50
31.8	107	60

$$a = 4(c*g) + 4\left(\frac{1}{2}*g^2\right) + c^2$$

$$c = g\sqrt{2}$$

$$a = 4(\sqrt{2} + 1)*g^2$$

$$d = 2g + c$$

$$d = g(2 + \sqrt{2})$$

$$a = \frac{4(\sqrt{2} + 1)}{(2 + \sqrt{2})} * d^2$$

$$\therefore a = .8284*d^2$$

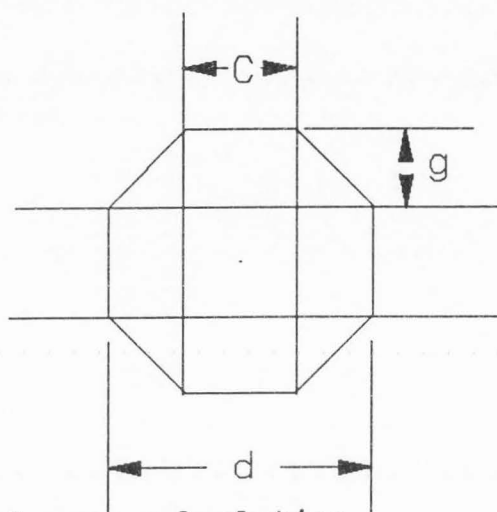


Figure 4. Bead area diagram and area calculation. Variables used in the calculations are those depicted in the diagram.

So the projected area of a single 6 mm bead is .2982 cm<sup>2</sup> and for an 8 mm bead is .5302 cm<sup>2</sup>.

#### Transmittance versus Bead Diameter and Color

Since the particles were much larger than 20x the wavelength used, the only expected interactions of significance were reflection and refraction. Particle properties that would have effected these interactions were particle size and optical characteristics. It was necessary to establish the dependancy of transmittance on particle size, the optical properties of the particle, or an interaction of both factors.

To test the transmittance/optical property relationship, a split-plot, randomized block design was implemented, with total area as the block and color

(representing optical properties) as the plot. There were three blocks in the experiment with eight samples in each block. The order of the blocks was randomized, as was the order of color of beads within each block. The entire experiment was replicated six times.

### **Curd Experiments**

#### **General Procedures**

Initially an experimental procedure was established to obtain valid results with the apparatus. In order to standardize the milk and obtain more universal results, Berridge substrate was used (12 grams of skim milk powder reconstituted in 100 ml of .01 M calcium chloride solution). The rennin used throughout the experiments was Hansen Standard Cheese Rennet. In all experiments the container and knife were placed in an incubator to warm to 30°C. The milk was warmed to 34°C in a hot water bath. It needed to be determined if the transmittance of coagulating milk could be followed with this apparatus. If the transmittance changes were large, the transmittance change would have to be factored into the measurements.

To retain exact whey-curd proportions, the exact volume of milk was coagulated in a small vat, 1 cm deep. Rennet was added to the milk, 450  $\mu$ l rennet to 2 liters of milk, and the milk poured into the vat and put back in the incubator at 34°C. After thirty minutes the gel was cut

and allowed to stand for 15 minutes to allow the gel to firm. The gel was carefully poured into the sample cell and the cell sealed. The cell was immediately placed in the apparatus to begin making measurements.

### **Effects of Whey on Light Transmittance**

As previously discussed, the optical characteristics of the whey change with time. This change had to be taken into account in the measurements. The absorption of the whey was measured with a spectrophotometer. The optical path length in the whey was the same in the sample cell and the spectrophotometer cell, simplifying measurement interpretation.

Maintaining the sample cell as a closed system was desirable. This precluded extracting whey from, or replacing extracted whey to, the sample. A procedure for determining the absorbance of the whey was established as follows.

After coagulating and cutting the substrate, 20 pieces of curd were put into the sample cell and the remainder of the volume was filled with whey from the batch. The cell was placed in the apparatus, rotating at 31 rpm. The batch vat was placed on a shaking table that oscillated slowly to approximate the agitation the sample was receiving. Samples of whey from both the sample and the vat were taken at:  $t=0, .5, 5$ , and 5.5 hours at which time the absorbance was measured.

### Initial Measurements of Syneresis

After determining what would not work with coagulation and whey sampling, a batch of substrate was coagulated and cut. The milk was inoculated with rennet and held for 20 minutes at 34°C. Then it was cut and the curd was allowed to firm for 15 minutes. The cell was filled with whey and 15 curds. The cell was placed in the apparatus and measurements were made every 15 minutes for the first hour, 30 minutes, and two measurements at one hour intervals:  $t = .25, .5, .75, 1.0, 1.5, 2.5, 3.5$  hours. In replications, measurements were made for 3 hours, every 10 minutes.

## CHAPTER V

### RESULTS AND DISCUSSION

#### Equipment Design

##### Sample Cell

A major problem with the initial design was that the rings, although machined round, would distort and twist when they were turned on tight enough to prevent leakage. Poor detection resulted from non-parallel end plates. The distorted rings also made the sample cell rotate non-parallel to the beam axis. This design did confirm that a long sample cell would result in multiple scattering and yield very low unscattered light transmission.

Problems with the second, shorter, cell design also included curd stacking and multiple scattering that yielded unreliable results. Interpretation of whey absorbance was cumbersome since the spectrophotometric cell and sample cells were different lengths.

The final cell was so thin that it drifted on the rollers. This did not affect the transmittance of the light beam, but was inconvenient. It also had no sample port. This meant a secondary sample had to be run to determine whey absorbance. The back up cell was the second cell. This cell contained the same proportion of curds and whey as the cell for which transmittance was measured. In order to preserve similar conditions between

the two cells, extracted whey was replaced immediately. This required the use of two syringes. First, whey was extracted and placed in the cuvette. Then one syringe was filled with whey, and while the whey was being replaced with one syringe, the air was extracted with the second.

The secondary cell did not leak under conditions of use, but when whey was extracted, air was incorporated into the cell. Simply replacing the whey resulted in increased internal cell pressure. Subsequently, whey was forced out of the cell. Since external curd pressures have a pronounced impact on syneresis, the air extracted as whey was added.

The cover plate retaining rings on both sample cells were built to be held in place by six bolts. Three bolts were adequate to achieve a leak proof cell. Bolts were tightened only slightly. Over-tightening the bolts resulted in the cover glass chipping and cracking.

### **Apparatus**

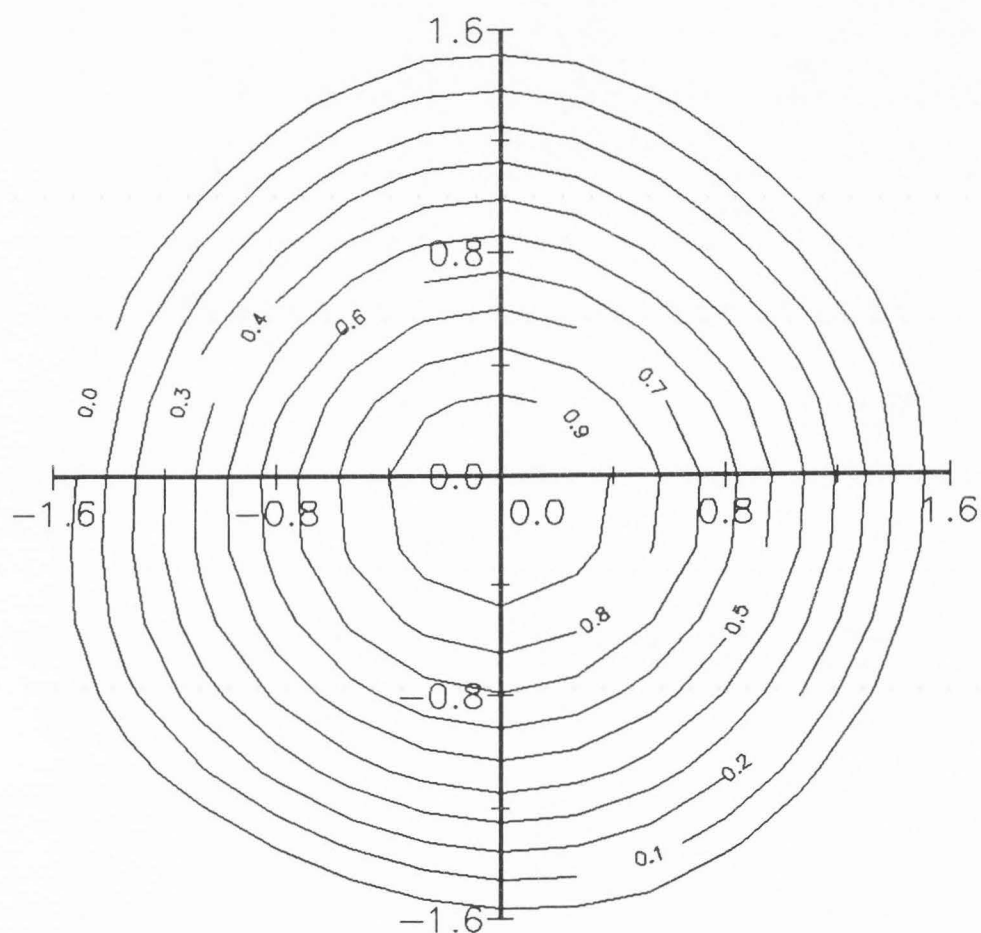
Even though the apparatus was built to factor out stray light, interference from external sources was expected. To reduce this interference the apparatus was built with rubber curtains over the front and the optics were enclosed. This design aspect was a success. With the curtains in place the detector did not pick up a significant amount of light ( $<10^{-8}$  the amount registered



when the laser is on.) When the curtains were opened, twice as much background light was detected.

To prevent scattered and background light from being measured a pinhole filter was placed at the focal point of the collector lens system. The acceptance cone of the filter was 138 mrad. This accepted 96% of the light diffracted around the edges of the curd. The maximum amount of stray or scattered radiation that was detected was less than .01 the value of the last significant digit of the measurement.

Since the laser beam was run through a series of lenses and expanded, the final profile of the beam had to be determined to ensure illumination was adequate. The measured intensity profile is depicted in Figures 5 and 6. Figure 5 is a contour plot of the beam power and Figure 6 is a three-dimensional depiction of the intensity profile. The beam profile was not flat, preserving the Gaussian shape of the original laser beam, but the distribution was fairly uniform. The important factors were that the entire surface of the sample cell received illumination and the beam power did not go to zero before reaching the edges of the sample cell. As curd distribution was random but consistent with time, the distribution of the power did not present difficulties. If the beam profile had a spike, there may have been problems with curd passing through the beam and registering excessive absorbance.



Expanded Beam Profile

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Figure 5. Contour plot of the normalized beam intensity. The center value is 1. The diameter is 3". Sampling was done by occluding all but a .25" diameter circle that was positioned at various points in the expanded beam.

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Normalized Profile of Beam Power

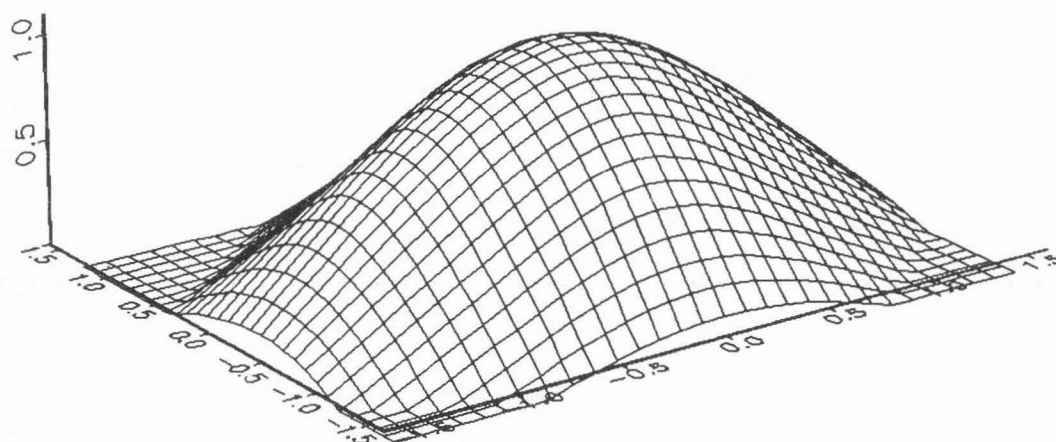


Figure 6. Three-dimensional depiction of beam intensity profile. Units on the x and y axis are inches, and the z axis is unitless. Note the Gaussian shape of the profile.

The laser output fluctuated throughout the day. The variability in power from the electrical outlet may have contributed to the fluctuation but there were no consistent trends from day to day as would be expected. Manufacturer's specifications include a 5% variability in the laser output after a 12 hour warmup. When the laser was warmed up, the detected light changed less than 1% in thirty minutes.

When the drive mechanism was turned on, detected light fluctuated as much as 10% between half second readings. At slow speeds (<20 rpm) the fluctuation was more pronounced, but as motor speed increased, fluctuation steadily decreased to 2%. Variability was more pronounced with the cell in place. The position of the cell had no effect on light transmittance. Even though the cell migrated along the axis as it rotated, the light detected did not change. When the cell was in place, variance in the readings oscillated within a 15% interval. To compensate for the fluctuation, at least 120 readings were taken, and the average was used as the measurement. The sample variance for each reading was less than .8%.

### **Calibration with Beads**

#### **Transmittance versus Total Bead Cross Sectional Area**

Sample data of transmittance corresponding to total bead cross section is tabulated in Appendix B, the results

are depicted graphically in Figure 16, and the analysis of variance is tabulated in Table 6. The statistical model for the split-plot, randomized block experiment that was conducted is:

$$T_{ijk} = \mu + R_i + S_j + \text{Error}(s) + A_k + S * A_{jk} + \text{Error}(w) \quad (21)$$

where  $T_{ijk}$  is sample transmittance,  $R_i$  is variability due to reps,  $S_j$  is variability due to bead size,  $A_k$  is variability due to total area and  $S * A_{jk}$  is the interaction variance.  $\text{Error}(w)$  and  $\text{error}(s)$  are the whole plot and subplot errors against which the other factors are tested.

The total area of the sample cell is 55.6 cm<sup>2</sup>. With 10% occlusion there is approximately 86% transmittance. Occluding 19% of the area permits about 73% transmittance. The exponential relationship between area and

**TABLE 6.** Statistical analysis of transmittance versus total particle cross sectional area and bead diameter. Run as a split-plot design with diameter as the whole plot, cross section as the subplot.

Source	DF	SS	MS	F	P
reps <sub>i</sub>	1	.000000	.000000	.00	
Size <sub>j</sub>	1	.001040	.001040	3.38	.70
Error(w)	1	.000308	.000308		
Area <sub>k</sub>	5	1.027250	.205450	870.55	<.001
S*A <sub>jk</sub>	5	.001180	.000236	.93	.502
Error(s)	10	.002541	.000254		
Total	23	1.032319			

transmittance as predicted by Beer-Lambert theory (see Figure 7) is not as good as the linear correlation predicted by Fraunhofer diffraction theory (see Figure 8).

### Transmittance versus Diameter and Optical Properties

Sample data is presented in appendix C. In this experiment  $T_{ijk}$  is the transmission value,  $R_i$  is the variability due to replication,  $S_j$ , the whole plot, is the particle size, Error(w) is the whole plot error.  $C_k$ , the subplot, is the color of the bead or the various optical characteristics analyzed,  $S*C_k$  is the size-optical interaction, and Error(s) is the subplot error.

The analysis of variance on the data is detailed in Table 7. From this analysis it is obvious the particle size is a significant factor. Optical characteristics of the beads, denoted by color, are not significant, nor was the interaction between size and color significant.

**TABLE 7.** Statistical analysis of diameter and optical characteristics versus transmittance.

Source	DF	SS	MS	F	P
Reps	5	.02286	.004572	16.92	
Size	2	1.19036	.595179	254.35	<.001
Error(w)	10	.02340	.002340		
Color	7	.00324	.000463	1.46	.120
S*C	14	.00520	.000371	1.37	.190
Error(s)	105	.03003	.000286		
Total	143	1.27508			

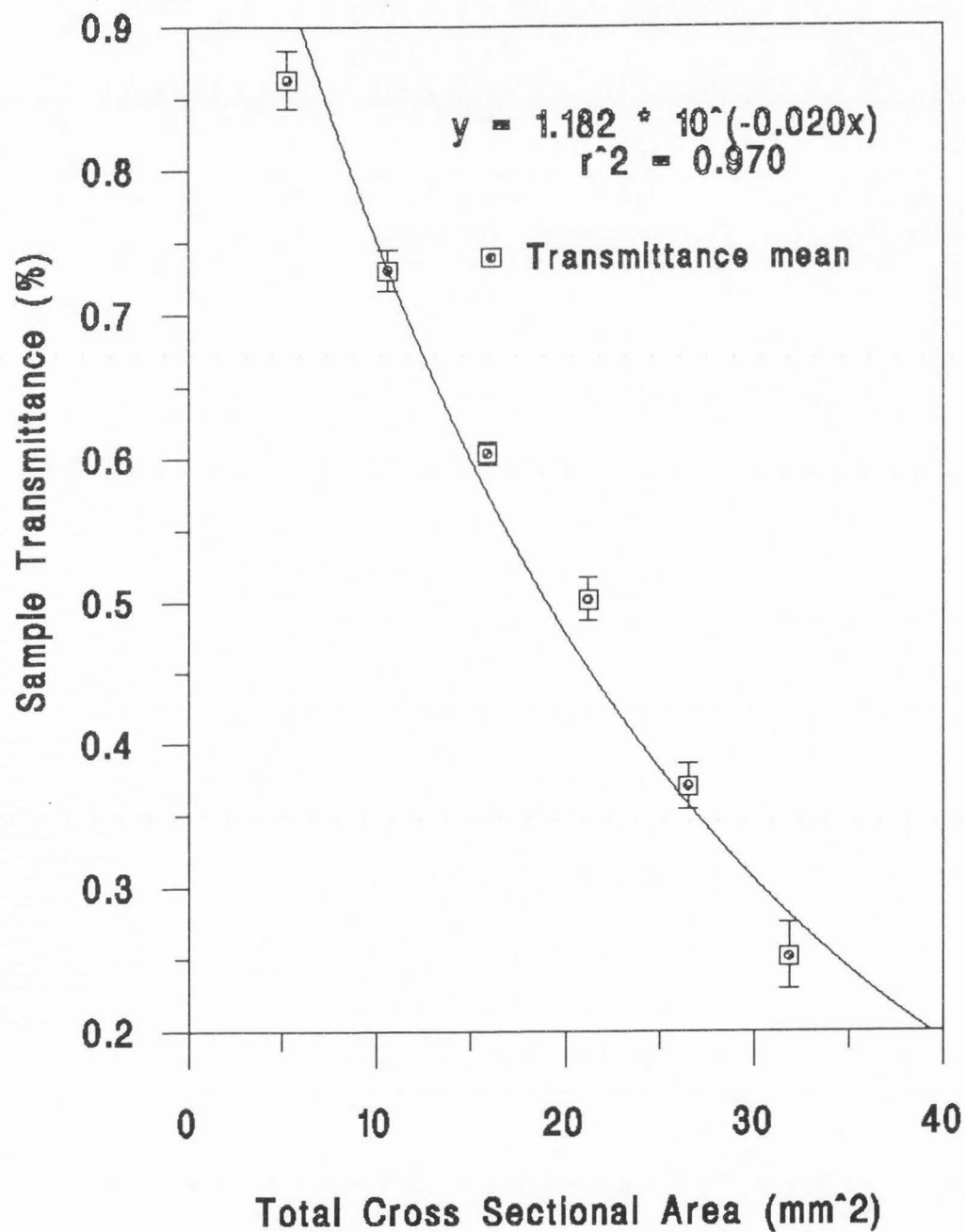


Figure 7. Logarithmic fit to bead sample transmittance. Data are fitted with an exponential curve as predicted by Beer-Lambert theory.



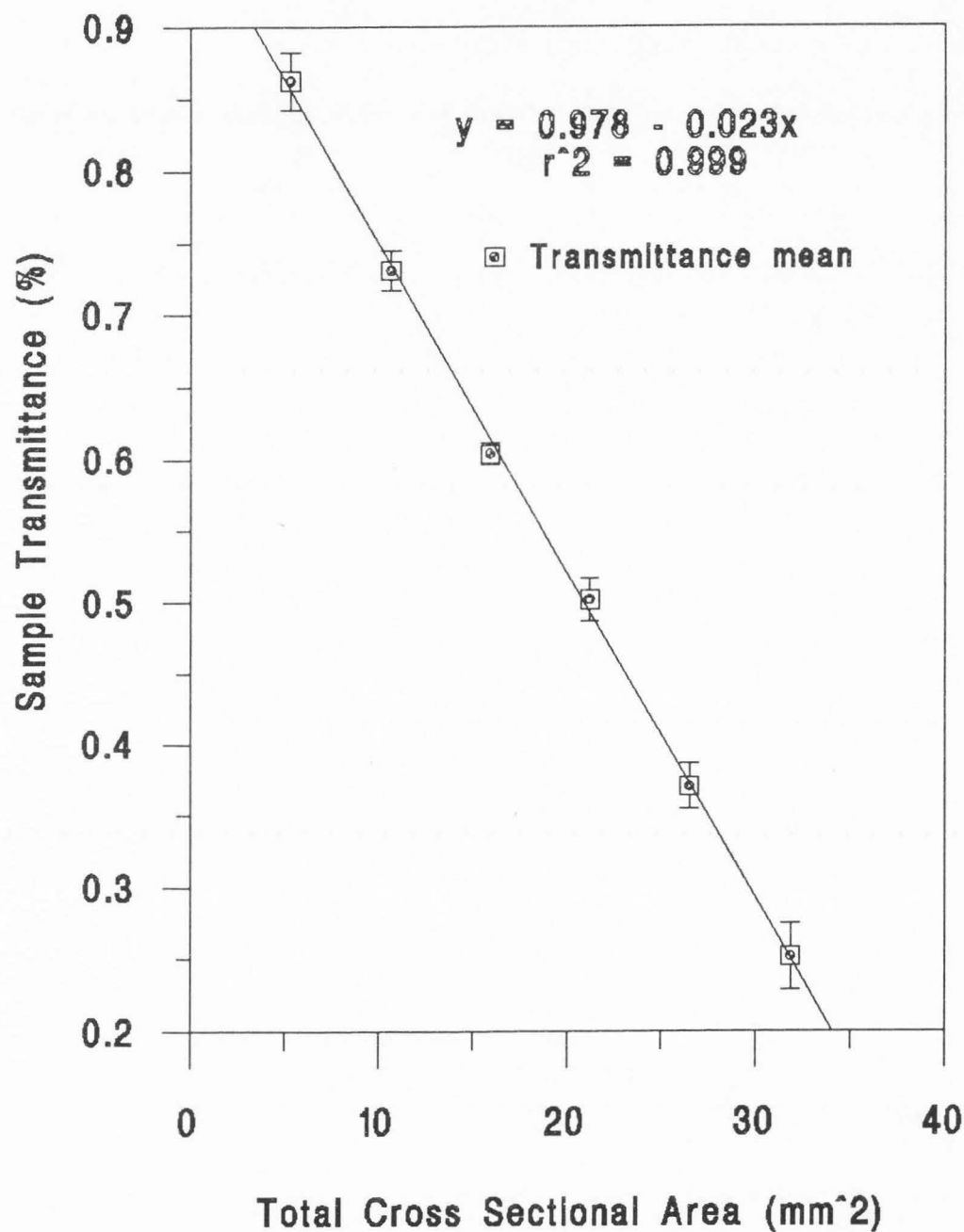


Figure 8. Linear fit to bead sample transmittance. Data fitted with a straight line as predicted by Fraunhofer diffraction theory, absorbance is proportional to cross sectional area.

Transmittance is dependent on total particle area, and particle size for a given number. The data from the total cross section experiment was used to calibrate and determine the equipment constants. The equation for the linear correlation between transmittance and bead size was derived as follows:

$$T = 0.978 - 0.023A_p \quad (22)$$

where  $T$ =transmittance and  $A_p$ =na.

### **Analysis of Curd**

#### **Effects of Whey on Transmittance**

The transmittance of the whey from the shaken vat decreased with time from an initial value of 74.8% to a final value of 28.0%. The whey from the vat had so much suspended particulate coagulum from shattered curd that measuring transmittance was difficult. The transmittance continually changed as particulate matter settled out of suspension. Running a secondary cell enabled transmittance measurement of whey that had been subjected to the same treatment as the curd sample. The curd was kept in suspension but directional changes were not as abrupt and the curd did not break apart. This technique yielded a curve that quickly approached an asymptote. See the comparison curves in Figure 9.

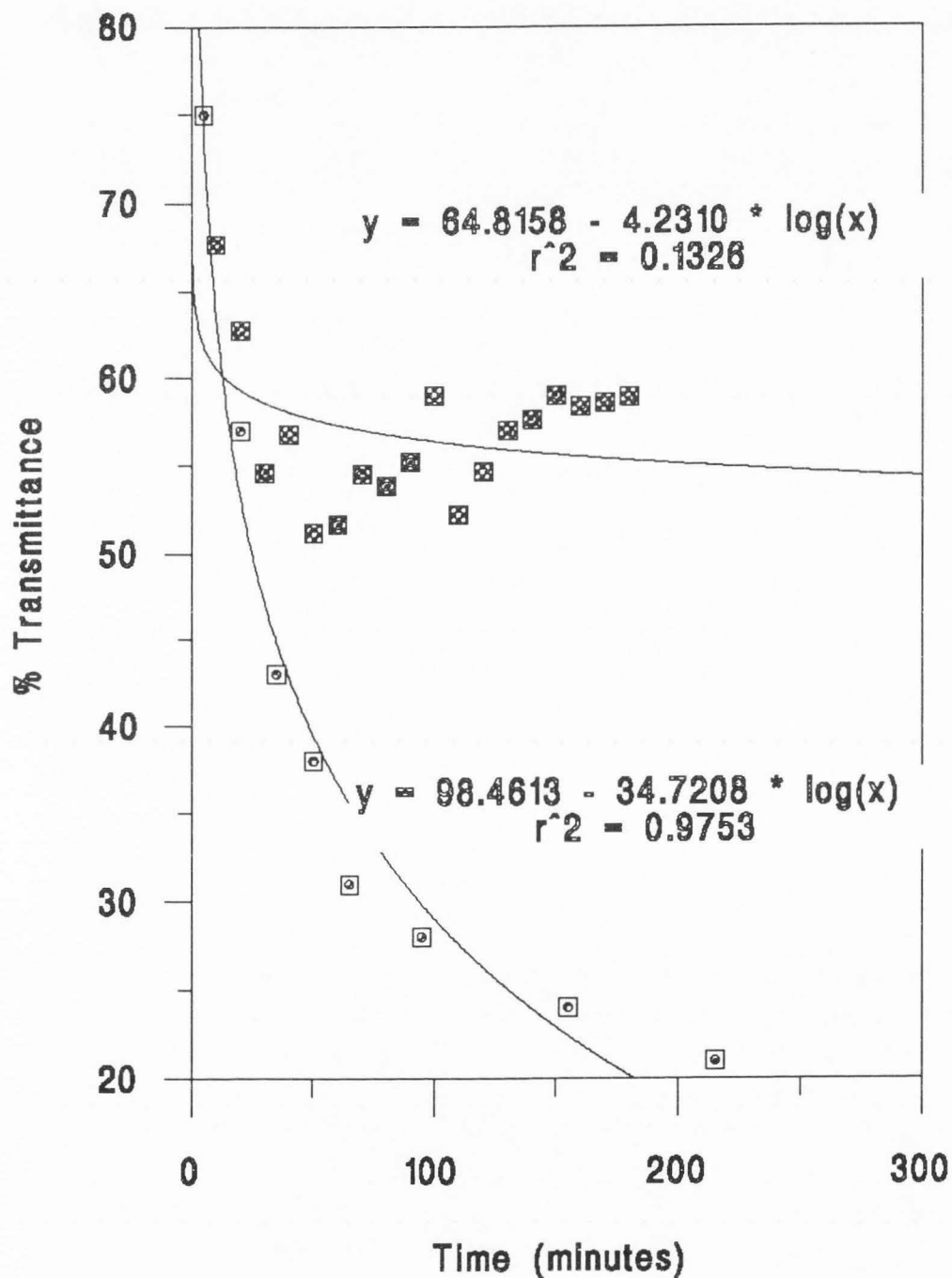


Figure 9. Transmittance of whey subjected to different treatments. The lower curve was transmittance of whey that was shaken. The upper curve was from a sample that was rotated in a duplicate cell.

### Coagulation and Syneresis

The change in transmittance as milk coagulates can be followed with a spectrophotometer. To test this apparatus's ability to filter out scattered light and transmittance, milk was coagulated in the cell and measurements were begun immediately. Rennet ( $15\mu\text{l}$ ) was added to 100 ml Berridge substrate that had been warmed to about  $30^{\circ}\text{C}$ . Coagulation was achieved in 8 minutes under these conditions as determined by the Formagraph method (65). After addition of the rennet to the sample, the sample cell was carefully sealed, ensuring that incorporated air was minimized. This sample was placed in the apparatus. There were no changes in the transmittance with time. After a time the cell was removed from the apparatus and syneresis induced.

Syneresis can be induced by either of two methods, cutting the curd or wetting the surface. Both of these methods were tried and failed. To observe one-dimensional syneresis, distilled water was layered onto the gel and allowed to stand for 30 minutes before sealing the cell to make measurements.

The amount of transmitted light did not change, and the curd did not synerese. After thirty minutes the curd was cut in an attempt to get the gel to synerese. The curd was cut into pieces 5 mm on a side. As before, the cell was sealed, taking care to prevent incorporating air,

and the transmission measured. There were no syneresis or transmittance changes. Although the apparatus was running at 28 rpm, the agitation was little or non-existent as the curd filled the sample container, adhered to the glass surfaces, matted back together, and rotated with the cell. The relative positions of the curd and the sample cell remained unchanged. Besides the theoretical restrictions, this is another reason why particle number density must be low. The curd had to be coagulated externally and transferred to the sample container.

#### **Initial Measurements of Syneresis**

When the exact volume of substrate was coagulated and transferred, the transmittance actually decreased over time. There was not enough whey in the container for the curd to be in suspension. The curd shattered, and small particulate coagulum filled the inter curd spaces.

With this as a guide, a two-liter batch of substrate was coagulated and cut. Fifteen curd were transferred to the cell and the remaining volume was filled with whey. The changes in the transmittance are presented in Figure 10 and calculated changes in curd size are presented in Figure 11. The transmittance of the curd sample increased with time as the curd shrank. When whey absorbance was factored out, the data became scattered. Many replications may isolate whey effects and enable the derivation of a mathematical model.

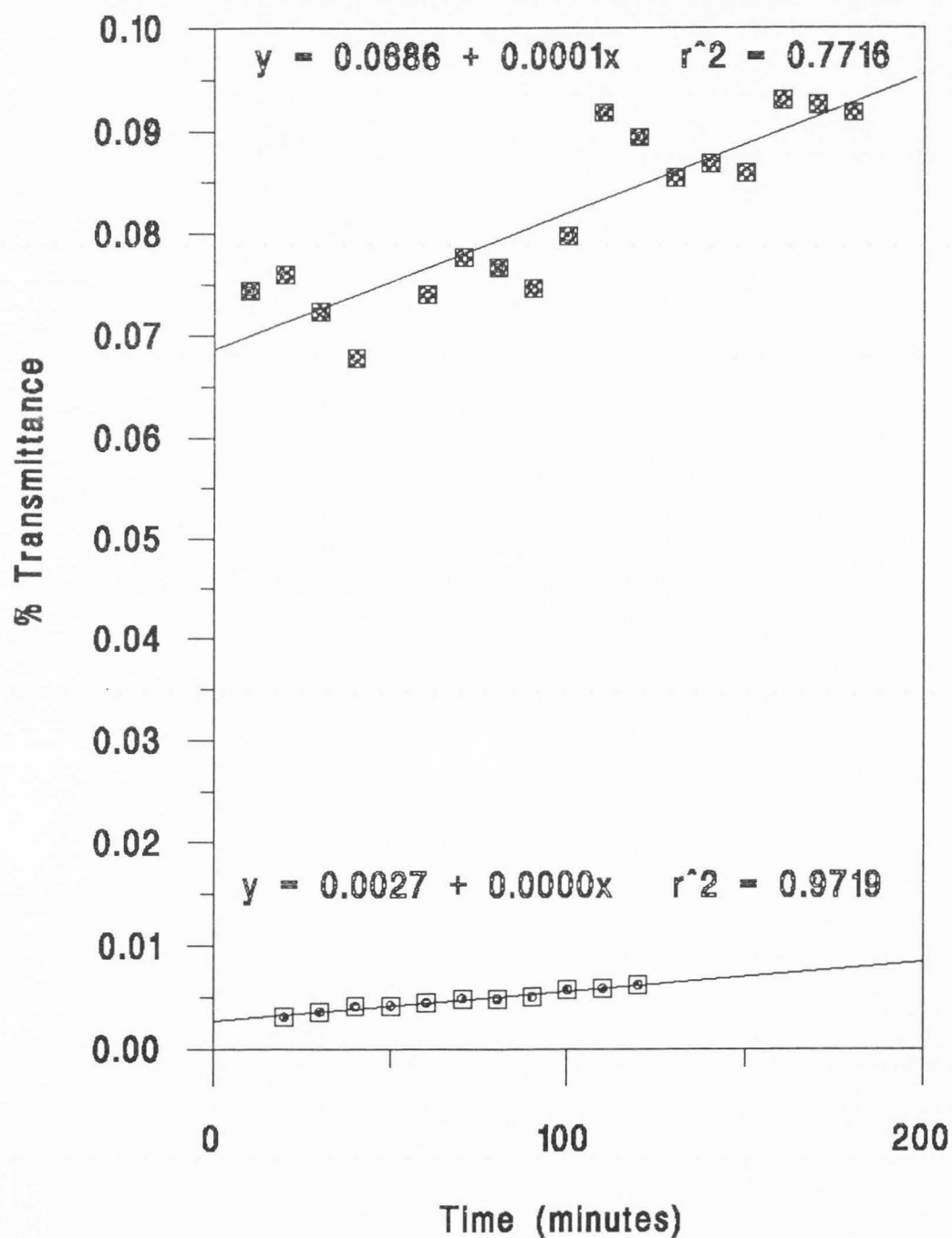


Figure 10. Measured changes in transmittance of curd sample. Absorbance due to the whey and sample cell are factored out.

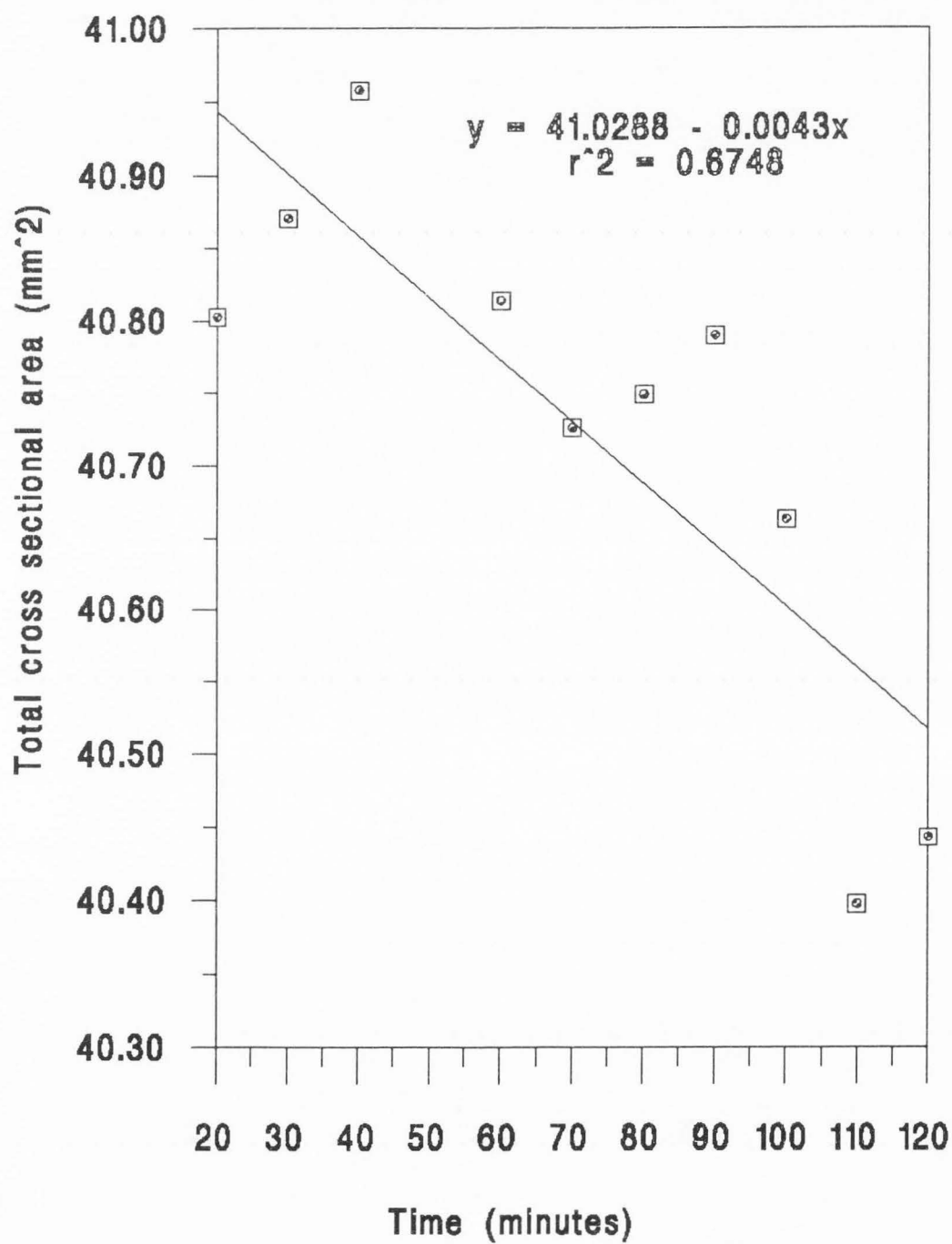


Figure 11. Changes in the cross sectional area of the curd calculated from transmittance values.



## CHAPTER VI

### CONCLUSIONS AND RECOMMENDATIONS

#### General Observations

The overall objective was to construct an instrument to evaluate syneresis in real time. To accomplish this several secondary objectives were outlined, namely 1) determine the necessary optical components and relative optimal spacing of these components, 2) design and calibrate the sample vial for the desired characteristics, 3) determine the optimal angle between sample and detector for collection of scattering data, 4) calibrate the equipment to establish the relationship between particle size and light transmission, and 5) make preliminary measurements of syneresis and establish an error variance.

The design of the apparatus has been discussed. The optical characteristics were established as being adequate by evaluation of the enlarged laser beam. The optimal measurement angle was determined to be  $0^\circ$  because of particle size and approximations that had to be made.

The apparatus is a success in that it does show that the size of macroparticles, such as curd, can be determined from apparent absorbance. The presented area of the particle can be correlated to the volume of the particle. All results corresponding to area effects on results were significant. There was a direct correlation between total

area and transmittance. The graphs of the transmittance of the bead samples show this strong correlation very well. Although the fit of an exponential curve, as predicted by Beer-Lambert theory, is very good, a simple line can be made to fit the curve better.

The difference between Fraunhofer diffraction theory and Beer-Lambert theory is the incorporation of multiple absorption layers. Without multiple layers the exponential aspect of absorption is not justified. The results from calibration using beads shows that the area presented to the beam by the particle is proportional to the amount of light absorbed. This is in agreement with Fraunhofer theory.

The results indicate that a significant portion of light is not transmitted directly through the curd without interaction. The pinhole filter is effective as it removes the background radiation and the light that interacts.

The distance between the curds must be fairly large for several reasons. The particle number density cannot exceed theoretical limitations, and the curds must have room for movement to permit agitation. This can be accomplished by making up large (1 liter) batches and filling the cell with whey, then transferring curd into the sample cell for measurement. The cell should be able to remain closed for the duration of the experiment.

### Limitations of the Method

Limitations of this method are that the sample cell must be short enough that particles cannot stack. If cell length permits stacking, curd size is predicted to be smaller than it actually is, and conditions of Beer-Lambert theory are introduced. Measurements cannot be made until the curd is sufficiently firm and whey separation has taken place. Measurement of the initial stages of syneresis is not possible with this technique. There must be a certain amount of whey available to put in the cell with the curd and keep the curd in suspension. The curd environment is somewhat artificial due to the volume of whey required. Also syneresis can be followed only as long as substantial whey is present. After several hours the curd comes to equilibrium in the whey and syneresis stops. In cheese manufacturing, continued syneresis is maintained by removing the whey, and finally pressing the curd. This apparatus will not measure syneresis to the final point.

There were some points of difficulty and some unexpected problems. One of these was the power fluctuation of the laser. Commercial spectrometers compensate for this type of inaccuracy by splitting the light beam. This requirement was not anticipated, so we compensated by making two measurements, one with and a second without the sample in the beam path. Further variations were observed once the drive system was started. This was probably due to

mechanical oscillations of the beam expander and detector induced by vibrations of the drive system. One of the major causes of this vibration was the reduction gears.

In addition the light measurements oscillated once the sample cell was in place. This is probably due to the inability to obtain exactly (within a wavelength) parallel surfaces in the machining and assembly of the cell. As the cell rotates, the non-parallel faces act as a lens, focusing the beam off the optical axis of the apparatus. This slightly tipped beam is then clipped by the filter. This difficulty was overcome by taking the average of many measurements.

Secondly, the absorbance characteristics of the whey needed to be measured separate from the sample. Another design could incorporate a second spectrophotometer for this purpose. These two instruments could be coupled to the same computer and achieve real-time analysis objective. Because of the power fluctuations, and changing properties of the whey, it was impossible to achieve real-time analysis of the particle size.

As with any method, to make it useful, some idea of the variability and error involved must be known. With this method there is plenty of room for error. The laser fluctuates, whey properties change, and even the manner that the sample cell is assembled may affect the results. The most obvious source of error is at the detector. The light

that does not reach the detector will not be detected. This is partly overcome by making many readings of the transmission at the time of measurement. Since the detector registers detected energy every half-second, it is easy and quick to make many readings. In the experiments conducted 120 readings were used to obtain an average value that was used as the intensity value. Average standard deviation for these sample sets was .8%.

Error due to fluctuations of laser output has been discussed as well as the solution. Another major source of variability is the changing whey. This is the greatest variance in the system. Primarily the absorbance of a secondary sample is measured. This duplicate originates from the same batch and is subjected to the same conditions as the primary sample. Assuming they are optically the same, the lag time between the sample reading and the measurement of the whey transmission is a factor. Ideally the absorbance of the whey from the duplicate cell is the same as that in the sample cell. Another factor lending error is that transmittance of the whey is measured on a different machine than the one used to measure the transmittance of the sample. Theoretically the transmittance would be the same. The transmittance of the whey can change as much as 5% within five minutes due to settling out of suspended particulate coagulum. The lag time must be minimized. If the operator is conscientious

and performs the measurement rapidly, the variability in spectrophotometer measurement of whey transmittance is within 4% on a given sample.

Compounded, the variability in the measurement may be 8%. This is half the error of most methods (8,78,79,97,117) and conditions of syneresis are closer to those involved in manufacture, namely the curd is suspended in whey and is agitated. Measurements are performed on the same curd several times without interacting with the curd. Syneresis is uninterrupted and the environment unchanged when the measurement is made.

#### **Possible Improvements**

The length of the laser cavity depends on temperature. As the laser warms, the cavity expands and changes the beam intensity. Also air convection around the laser tends to cool the laser, changing the light output. The electrical input to the laser has a direct effect on the output. As the power comes directly from a conventional outlet, the power input fluctuates through the day. Both of these problems could be compensated for by incorporating a power output regulator in the design. An output regulator splits out a portion of the beam, and through a feedback loop, controls electrical input to maintain constant beam power. Many lasers, typically with higher output, incorporate a regulator in the laser. The solution used in this apparatus



was to make a measurement of the laser output immediately prior to or following a sample measurement. Another improvement would be the implementation of a beam splitter to obtain both a sample beam and reference beam. This would enable immediate calculation of sample transmittance. Even though the beam passing through the sample cell would be expanded, all that is really needed is a reference point of the laser power output.

A contributing factor to transmittance variability was the drive system. The drive system was mounted on the same table as the optics. Vibrations due to the drive system caused the focal point to move on the filter. It also caused the optics to drift out of focus, which required time-consuming readjustments.

A major improvement would be the integration of a second spectrophotometer to measure whey characteristics. The absorbance characteristics of the whey need to be measured separate from the sample. These two instruments could be coupled to the same computer and achieve the real-time analysis objective. The technology is in place to adapt these changes.

#### **Further Research Possibilities**

This apparatus could be used to model syneresis and give researchers a way to accurately predict syneresis. Characteristics of the milk could be measured, the model



applied, and properties of the product accurately controlled. This apparatus could be used with any system of large particles where the optical properties of the particles differed from those of the solution.

Industrially this apparatus could be implemented as a quality-control device in any application where particle size may be important. Currently continuous heating and pasteurizing operations are hindered because of the inability to accurately measure particle size in the flow. If the size of the particles changes with time, this change could be accurately measured and adjustments made. It could be used in biological systems where the activity of the organism produces a floc or a gel. These are but a few suggestions for further research and implementation of this apparatus and technology.

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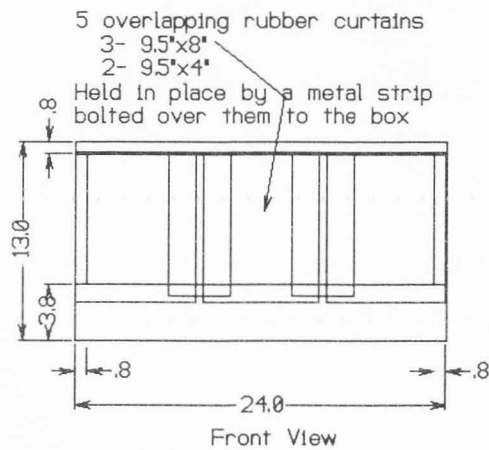


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**APPENDICES**



**APPENDIX A****Diagrams of the Design and Construction  
of the Apparatus**



Both ends have 4" holes cut in them  
 in them on the centers indicated  
 Also, 4 body holes for 1/4" bolts  
 on a concentric 6" diameter.

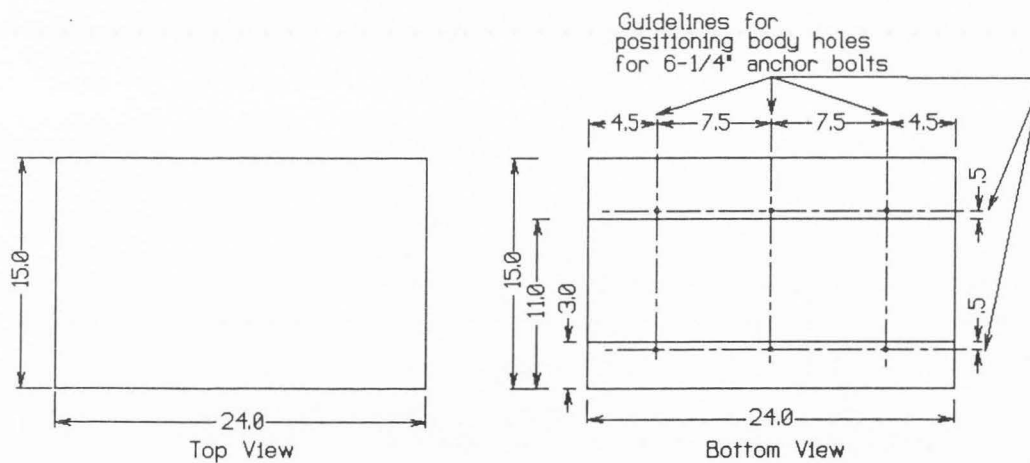
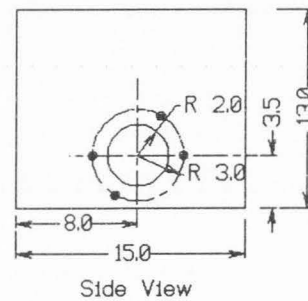


Figure 12. Experiment housing. The box is made of sheet metal and the curtains are rubber. It's held in place on the table by six 1/4" bolts through the bottom.

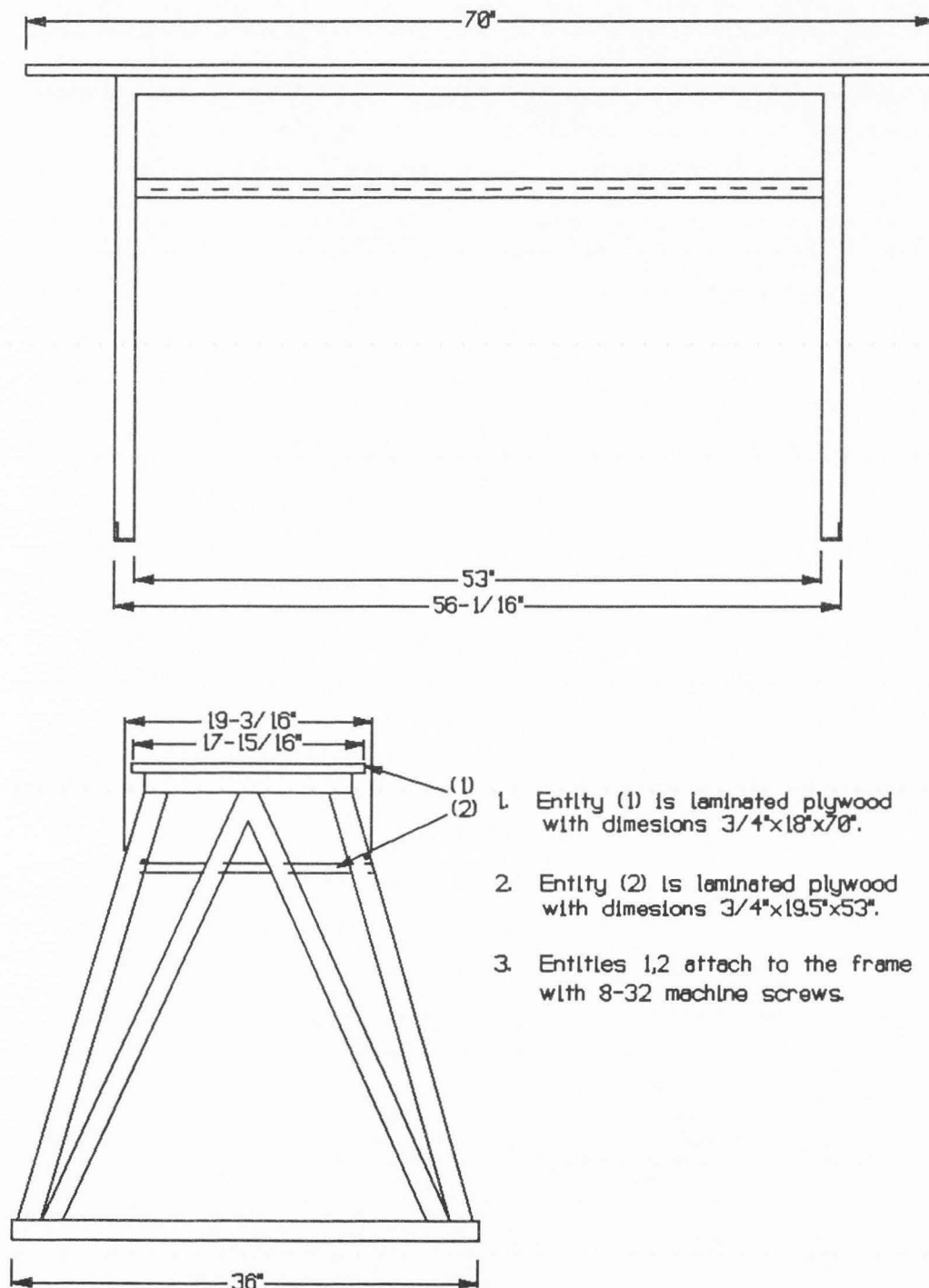


Figure 13. Mounting table. All members are stainless steel angle, 1.5" on a side by 3/16" thickness. Mounting surfaces are 3/4" laminated plywood fastened to the steel support members with 8-32 machine screws.

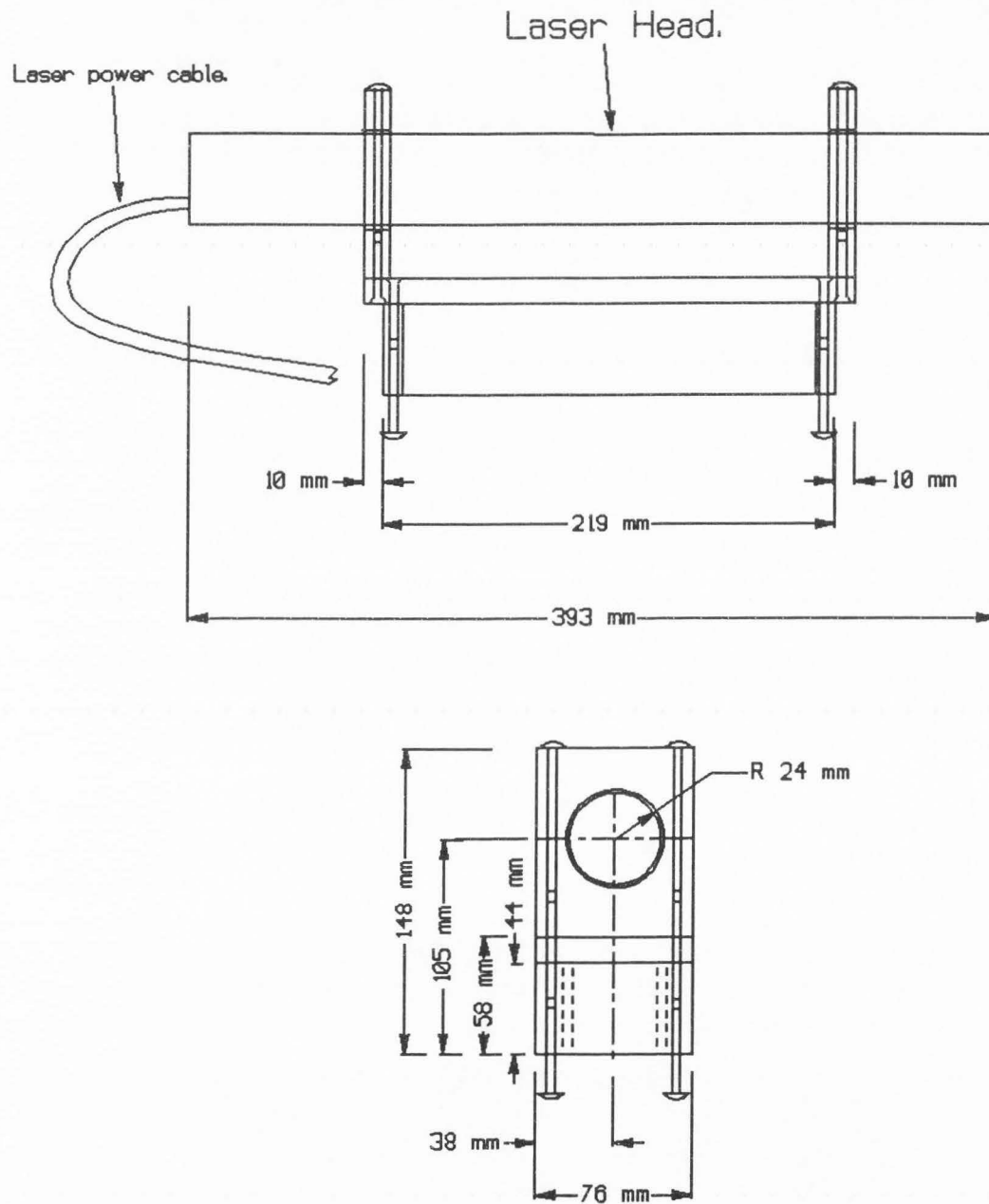


Figure 14. Laser head and mount. The support members are aluminum, mounting surface and clamping members are acrylic.

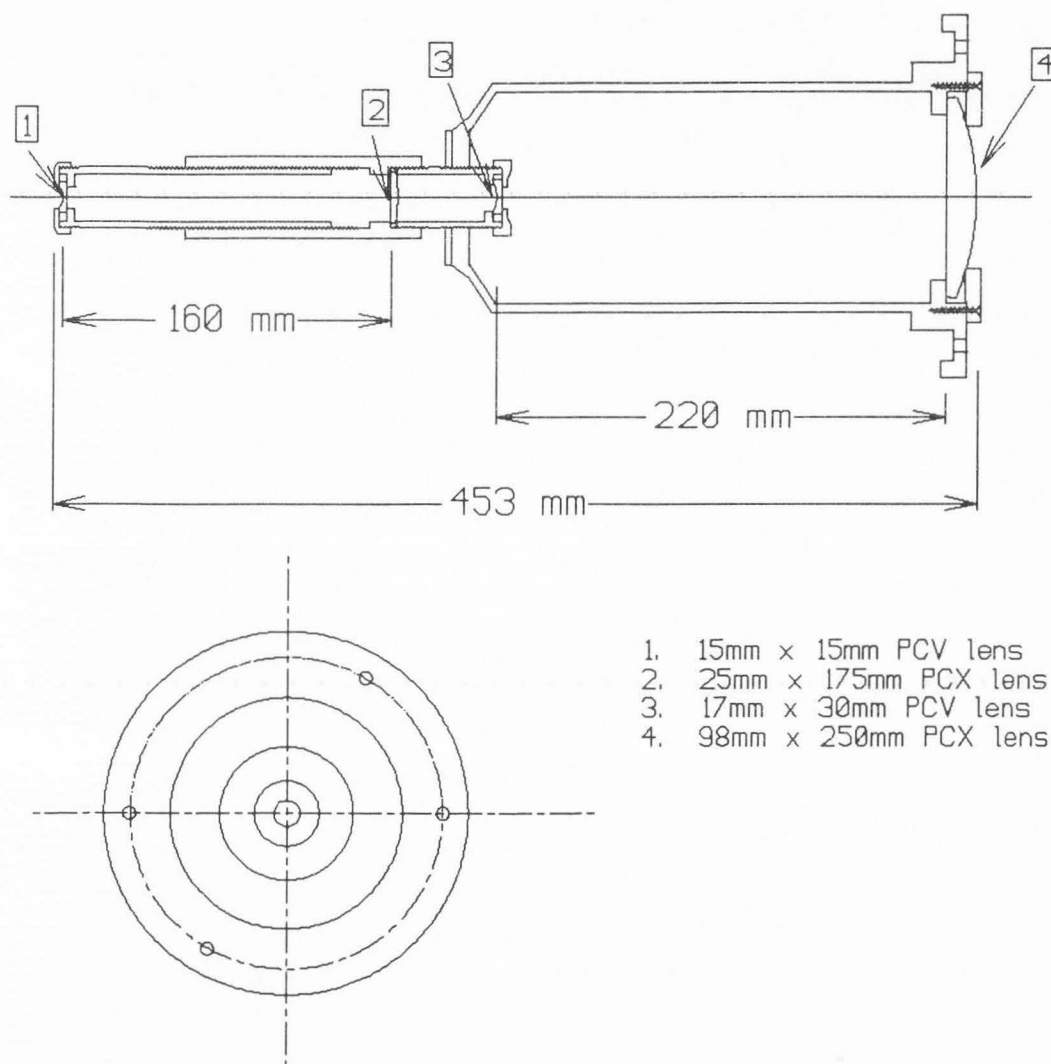


Figure 15. Beam expander. All components are made from polyvinyl chloride with the exception of the lenses. Expander achieves a 90 fold beam enlargement.

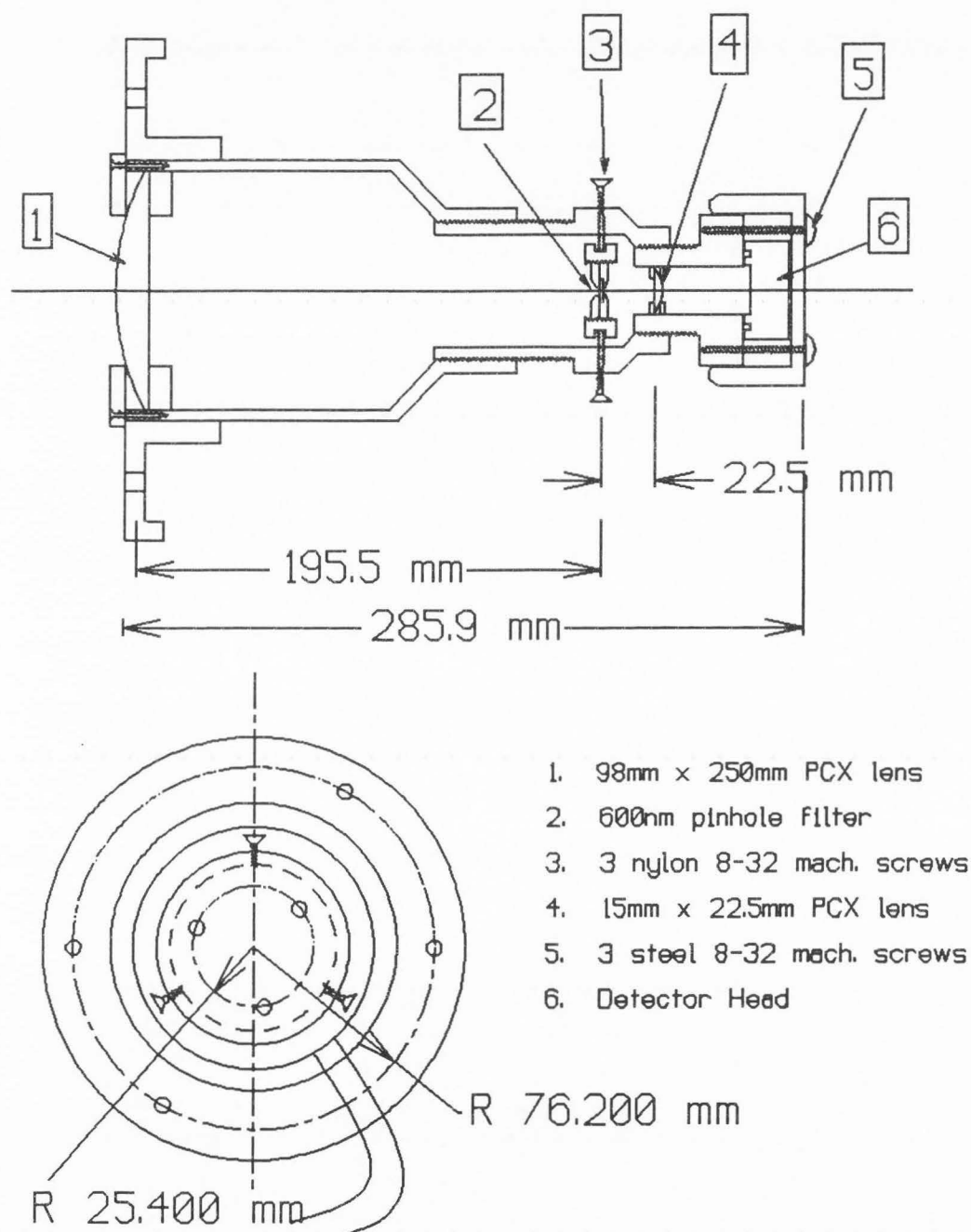


Figure 16. Light collector and detector. Members are constructed from poly-vinyl chloride. The filter and detector are centered and held in place by 8-32 machine screws.

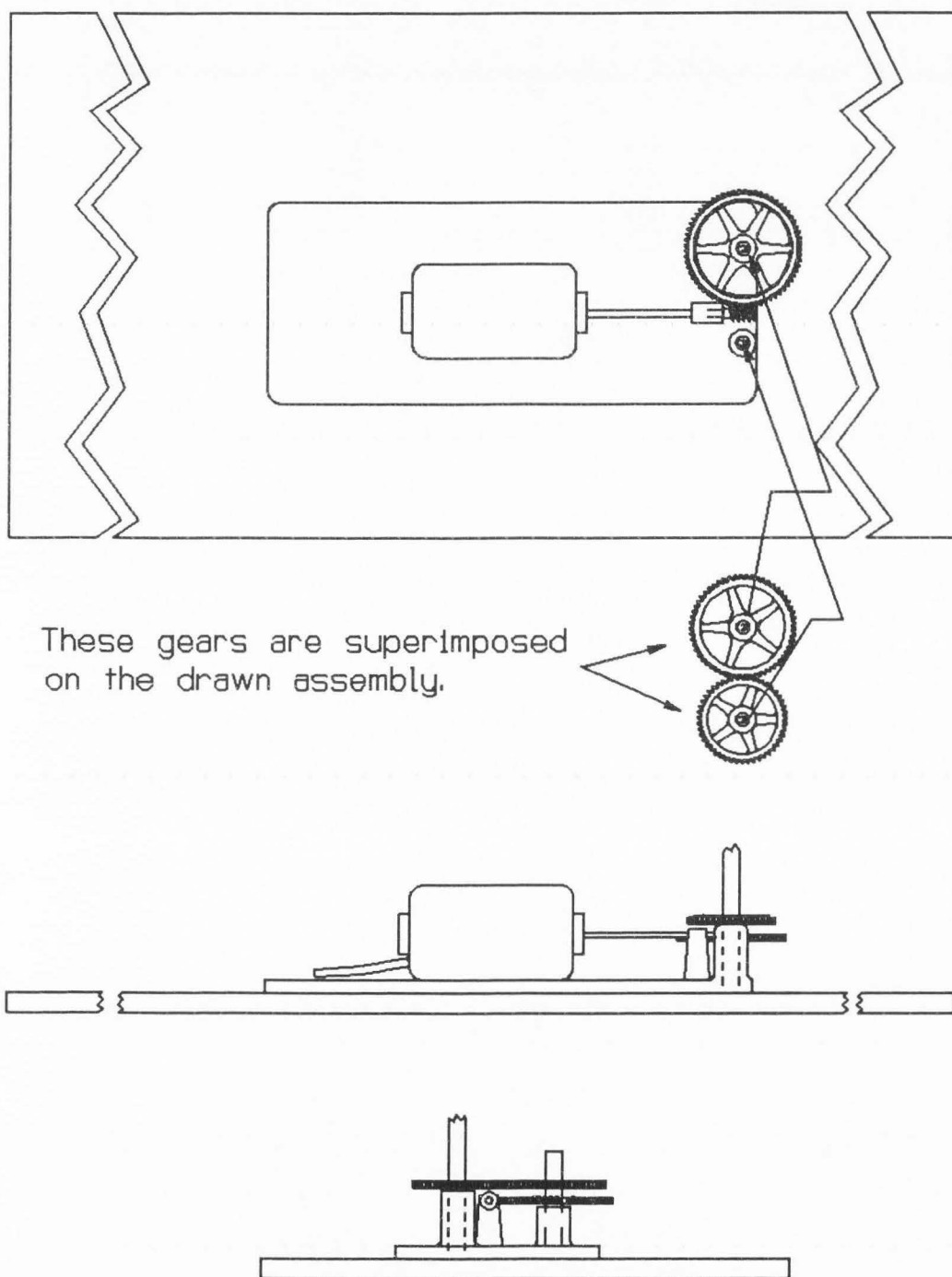


Figure 17. Lower drive assembly. Motor is a variable speed motor that runs up to 7500 rpm. Reduction gears shown have a 150:8 ratio. This drive assembly is mounted on the lower shelf of the table.



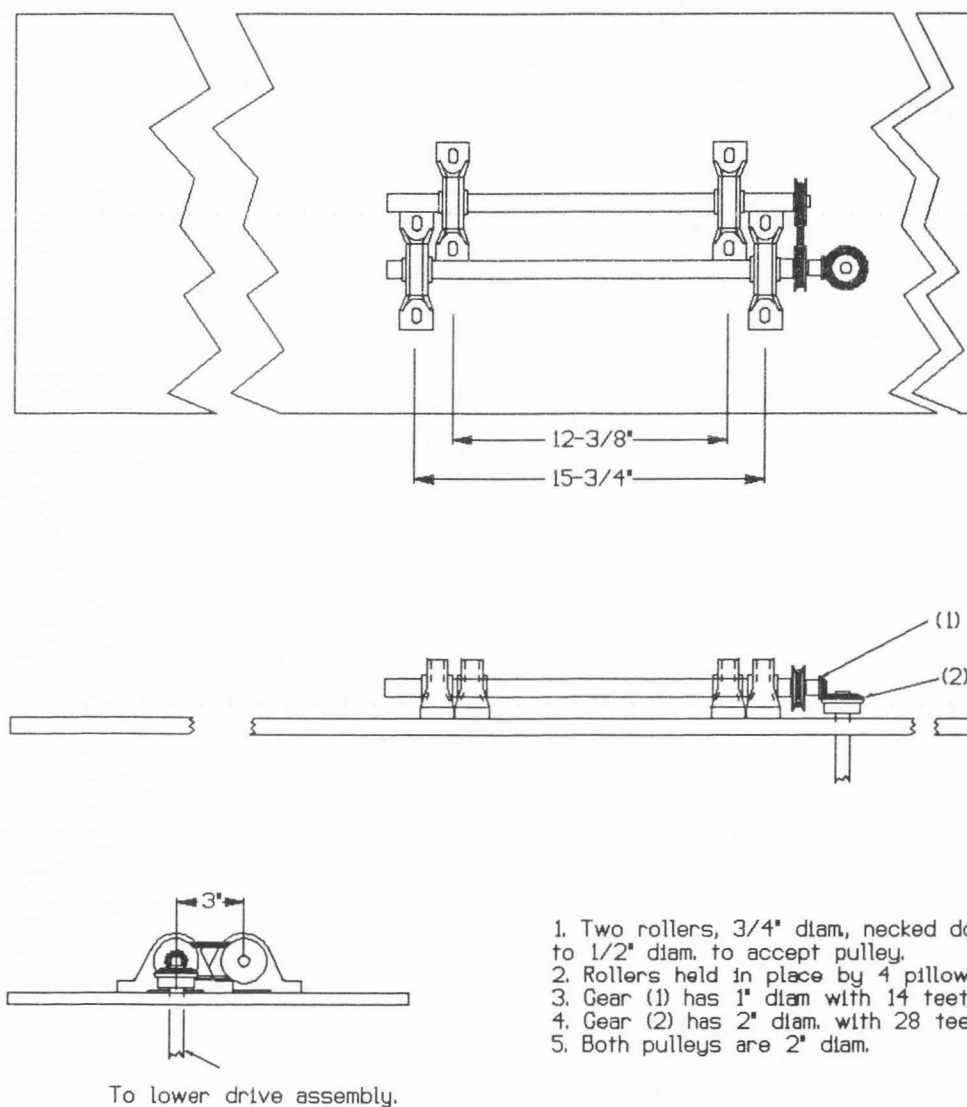


Figure 18. Upper drive assembly. The sample cell rests on two  $\frac{3}{4}"$  stainless steel rollers. Both rollers are powered, the second being belt driven from the other. This apparatus is mounted on the top shelf of the table.

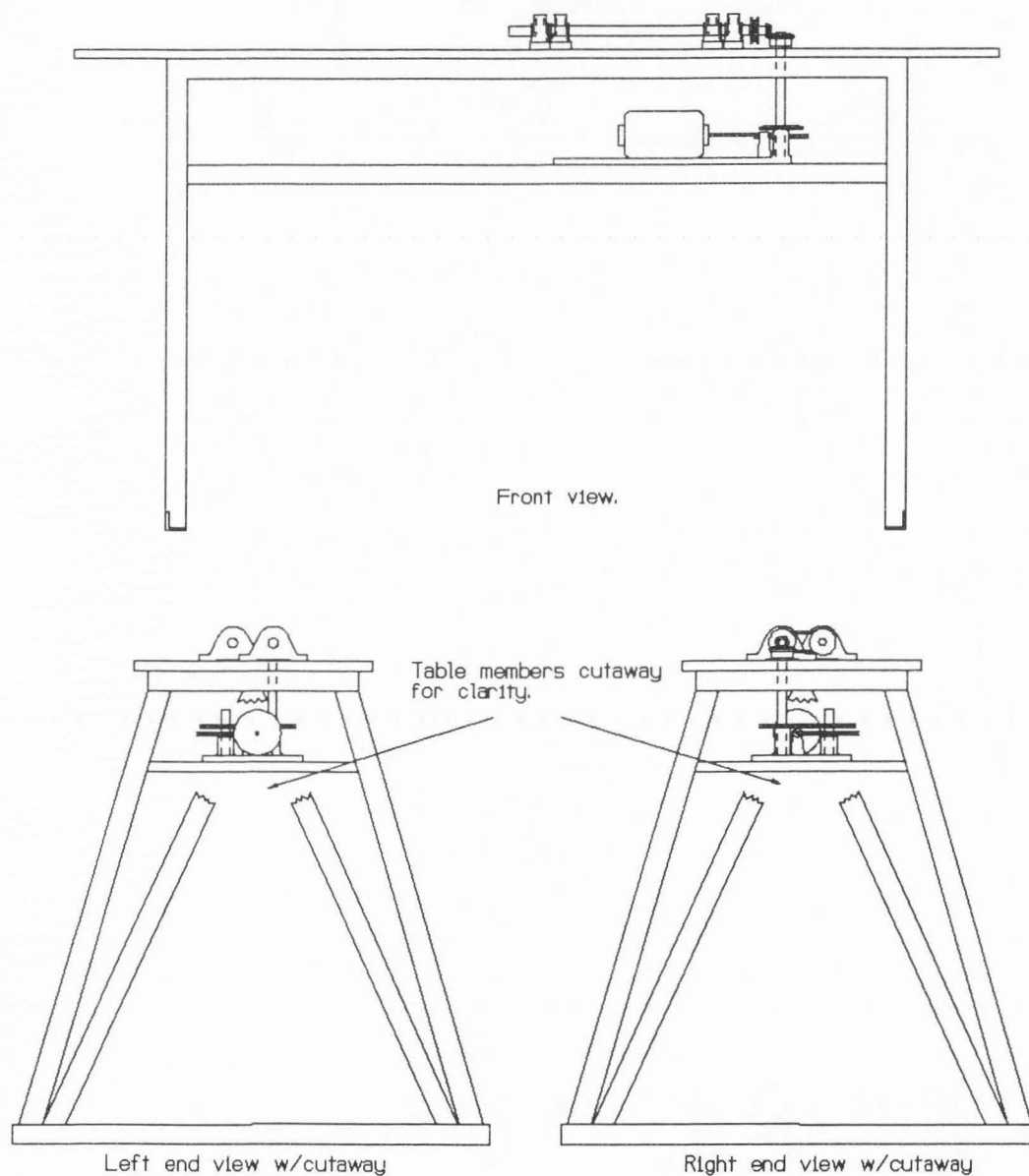


Figure 19. Complete drive assembly. Shows the assembly of the drive system. Table members cut away for unimpeded view of the drive system.

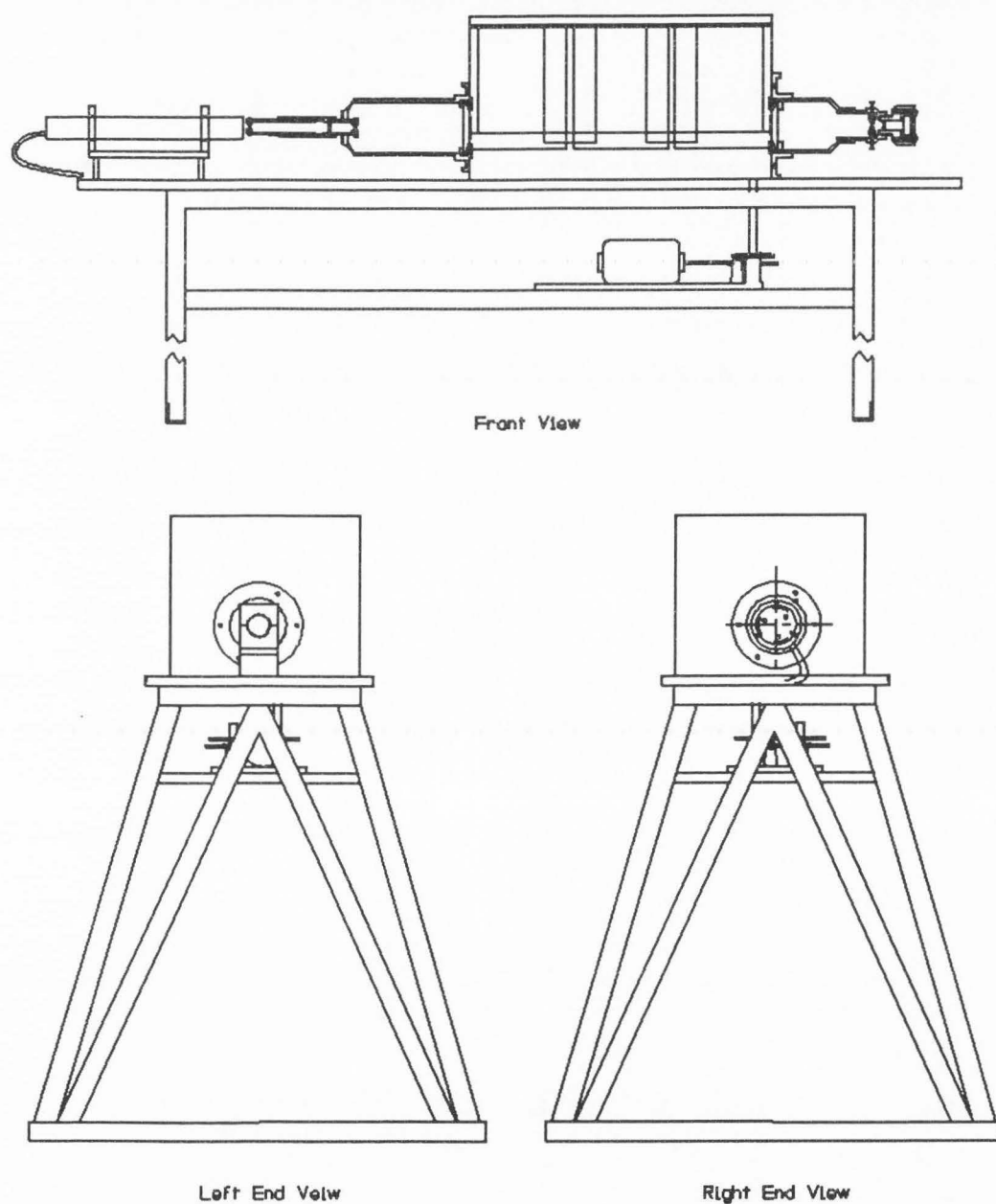


Figure 20. Complete apparatus assembly. Shows assembly of all components. Note the light path is totally enclosed when properly assembled.

## APPENDIX B

Sample Data from Transmission versus  
Total Cross Sectional Area

The numerical results are tabulated below:

<u>Cross Section(cm<sup>2</sup>)</u>	<u>Bead Diameter</u>			
	<u>6 mm</u>		<u>8 mm</u>	
	<u>Run 1</u>	<u>Run 2</u>	<u>Run 1</u>	<u>Run 2</u>
5.3	.890	.845	.851	.865
10.6	.720	.736	.749	.719
15.9	.601	.595	.606	.615
21.2	.488	.495	.523	.50
26.5	.381	.347	.382	.372
31.8	.224	.243	.272	.269

## APPENDIX C

Sample Data from Transmission versus  
Bead Size and ColorReplication 1

<u>Bead Color</u>	<u>4 mm</u>	<u>6 mm</u>	<u>8 mm</u>
Black	.8617	.7962	.6706
Brown	.8550	.7645	.6760
Clear	.9277	.7657	.6770
Cream	.9075	.8644	.6608
Solid Red	.8987	.7993	.6898
T. Green	.8736	.7501	.6615
T. Red	.8211	.8062	.6727
White	.8781	.8136	.6814

Replication 2

<u>Bead Color</u>	<u>4 mm</u>	<u>6 mm</u>	<u>8 mm</u>
Black	.9434	.7758	.6672
Brown	.9147	.7727	.6791
Clear	.9534	.8037	.6947
Cream	.9720	.7775	.6774
Solid Red	.9346	.7671	.6499
T. Green	.9233	.7832	.6788
T. Red	.9430	.8196	.6684
White	.9648	.7716	.6780

Replication 3

<u>Bead Color</u>	<u>4 mm</u>	<u>6 mm</u>	<u>8 mm</u>
Black	.9154	.7917	.6446
Brown	.9154	.7989	.6692
Clear	.9067	.8010	.6626
Cream	.8721	.8018	.6635
Solid Red	.9120	.7836	.6321
T. Green	.8711	.7787	.6536
T. Red	.8851	.8022	.6749
White	.9117	.7800	.6471

Replication 4

<u>Bead Color</u>	<u>4 mm</u>	<u>6 mm</u>	<u>8 mm</u>
Black	.9105	.7952	.7127
Brown	.9000	.8173	.7052
Clear	.8992	.8076	.7102
Cream	.9085	.8067	.6897
Solid Red	.9116	.8001	.6888
T. Green	.9216	.8213	.7042
T. Red	.9160	.8181	.6877
White	.9100	.8120	.6885

Replication 5

<u>Bead Color</u>	<u>4 mm</u>	<u>6 mm</u>	<u>8 mm</u>
Black	.8677	.8054	.6572
Brown	.8775	.8188	.6756
Clear	.9007	.7912	.6944
Cream	.9106	.7867	.6685
Solid Red	.9095	.7847	.6805
T. Green	.9052	.7962	.6876
T. Red	.8704	.7812	.6594
White	.9069	.8194	.6813

Replication 6

<u>Bead Color</u>	<u>4 mm</u>	<u>6 mm</u>	<u>8 mm</u>
Black	.9106	.8287	.6779
Brown	.9085	.8133	.7227
Clear	.9156	.8201	.7321
Cream	.9096	.8196	.7344
Solid Red	.9055	.8159	.7159
T. Green	.9002	.8186	.7342
T. Red	.9055	.8133	.7167
White	.9151	.8363	.7173



## APPENDIX D

## Initial Curd Data

<u>Time</u> (min)	<u>Whey</u> <u>Transmission</u> (%)	<u>Curd</u> <u>Transmittance</u>	<u>Curd</u> <u>Absorbance</u>
5	75	.8859	4.053
20	57	.9247	4.034
35	43	1.294	3.888
50	38	1.572	3.804
65	31	1.914	3.718
95	28	2.438	3.613
155	24	2.695	3.569
215	21	3.179	3.498

## VITA

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Commissioned Officer - Utah National Guard.  
July 1987 to present.

- Rank 1st lieutenant. Command as many as 100 people.

Laser Technician - Advanced Holographics, Logan, UT.  
May 1987 to December 1987.

- Developed and assembled optics for high resolution holograms.
- Monitored systems and production for quality.